(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 May 2001 (17.05.2001)

PCT

(10) International Publication Number
WO 01/35317 A1

(51) International Patent Classification[7]: G06F 19/00

(21) International Application Number: PCT/US00/31152

(22) International Filing Date: 13 November 2000 (13.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/165,086	12 November 1999 (12.11.1999)	US
60/165,124	12 November 1999 (12.11.1999)	US
60/179,531	1 February 2000 (01.02.2000)	US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
- *With international search report.*
- *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/35317 A1

(54) Title: DETERMINING THE FUNCTIONS AND INTERACTIONS OF PROTEINS BY COMPARATIVE ANALYSIS

(57) Abstract: The invention provides novel methods for characterizing the function of nucleic acids and polypeptides. The invention provides a novel method for identifying a nucleic acid or a polypeptide sequence that may be a target for a drug. The invention provides a novel method for identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism. The characterization is based on use of methods of the invention comprising algorithms that can identify functional relationships between diverse sets of non-homologous nucleic acid and polypeptide sequences. The invention provides a computer program product, stored on a computer-readable medium, for identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism. The invention provides a computer program product, stored on a computer-readable medium, for identifying a nucleic acid or a polypeptide sequence that may be a target for a drug. The invention provides a computer system, comprising a processor and a computer program product of the invention.

DETERMINING THE FUNCTIONS AND INTERACTIONS OF PROTEINS BY COMPARATIVE ANALYSIS

Related Applications

The present application is a continuation-in-part application ("CIP") of Patent Convention Treaty (PCT) International Application Serial No: PCT/US00/02246, filed in the U.S. receiving office on January 28, 2000, and this application claims the benefit of priority under 35 U.S.C. § 119(e) of U.S. Provisional Application Nos. 60/165,124, and 60/165,086, both filed November 12, 1999, and U.S. Provisional Application No. 60/179,531, filed February 1, 2000. International Application Serial No: PCT/US00/02246 claims the benefit of priority under 35 U.S.C. § 119(e) of U.S. Provisional Application Serial No. 60/117,844, filed January 29, 1999, U.S. Provisional Application Serial No. 60/118,206, filed February 1, 1999, U.S. Provisional Application Serial No. 60/126,593, filed March 26, 1999, U.S. Provisional Applications Serial No. 60/134,093, filed May 14, 1999, and U.S. Provisional Application Serial No. 60/134,092, filed May 14, 1999. Each of the aforementioned applications is explicitly incorporated herein by reference in their entirety and for all purposes.

TECHNICAL FIELD

This invention generally relates to genetics and microbiology. The invention provides novel methods to identify the function of and relationships between nucleic acid and protein sequences. The method is particularly useful for finding the identifying genes and polypeptides having potential therapeutic relevance in organisms, e.g., microorganisms, such as *Mycobacterium tuberculosis*. The invention also provides *Mycobacterium tuberculosis* genes and polypeptides found by these methods. These genes and polypeptides are useful as potential drug targets.

BACKGROUND

The determination of the functions of and relationships between nucleic acid and protein sequences has traditionally relied on either the study of homology and sequence identity with genes and proteins of known function or, in the absence of informative homology, laborious experimental work. The availability of many complete genome sequences has made it possible to develop new strategies for computational determination of protein functions. Several methods have been developed which can predict the general

function of proteins by analyzing their functional relationships rather than sequence similarity. Generally, two proteins can be considered functionally related when they form part of the same biochemical pathway or biological process. For example, although malate dehydrogenase is not homologous to pyruvate carboxylase, and the two enzymes do not catalyze the same reaction, they are functionally related because they both catalyze steps of a common biochemical pathway, namely the tricarboxylic acid cycle.

New methods that can establish such functional relationships could provide valuable information on the functions of uncharacterized nucleic acid and protein sequences.

The disease tuberculosis, caused *Mycobacterium tuberculosis* (MTB) is one of the world's leading killers. The World Health Organization estimates that 30 million deaths from pulmonary tuberculosis will occur during this decade. Alarming reports on the emergence of drug-resistant strains of this bacterium underscore the importance of the search for new therapeutic agents. Identifying the function of every protein produced by MTB will provide researchers with promising new targets for anti-tuberculosis drug design.

SUMMARY

The invention provides novel methods for characterizing the function of nucleic acids and polypeptides. The invention provides a novel method for identifying a nucleic acid or a polypeptide sequence that may be a target for a drug. The invention provides a novel method for identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism. The characterization is based on use of methods of the invention comprising algorithms that can identify functional relationships between diverse sets of non-homologous nucleic acid and polypeptide sequences. Characterization of nucleic acid and protein sequences can be the basis for the development of compositions that can interact with those nucleic acids and polypeptides. For example, such characterization can provide a basis for screening methods. Such characterization may allow use of these sequences as targets for drug discovery. Discovery of such compositions can provide the basis for the design of novel drugs, particularly if the characterized sequences are derived from a pathogen.

The invention provides a method for identifying a nucleic acid or a polypeptide sequence that may be a target for a drug comprising the following steps: (a)

providing a first nucleic acid or a polypeptide sequence that is known to be a drug target; (b) providing at least one algorithm selected from the group consisting of a "domain fusion" method, a "phylogenetic profile" method and a "physiologic linkage" method, wherein the algorithm is capable analyzing a functional relationship between nucleic acid or polypeptide sequences; and, (c) comparing the first nucleic acid or the polypeptide drug target sequence to a plurality of sequences using at least one of the algorithms as set forth in step (b) to identify a second sequence that has a functional relationship to the first sequence, thereby identifying a nucleic acid or a polypeptide sequence that may be a target for a drug.

The invention provides a method for identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism comprising the following steps: (a) providing a first nucleic acid or a polypeptide sequence that is known to be essential for the growth or viability of an organism; (b) providing at least one algorithm capable analyzing a functional relationship between nucleic acid or polypeptide sequences selected from the group consisting of a "domain fusion" method, a "phylogenetic profile" method and a "physiologic linkage" method; and, (c) comparing the first nucleic acid or the polypeptide sequence to a plurality of sequences using at least one of the algorithms as set forth in step (b) to identify a second sequence that has a functional relationship to the first sequence, thereby identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism.

In one aspect of the methods of the invention, the drug is an anti-microbial drug. In another aspect, the first nucleic acid or a polypeptide sequence is derived from a pathogen. The pathogen can be a microorganism, such as *Mycobacterium tuberculosis* (MTB).

The plurality of sequences used to identify a second sequence can comprise a database of the gene sequences of an entire genome of an organism. The plurality of sequences used to identify a second sequence can comprise a database of the gene sequences derived from a pathogen.

In one aspect of the methods of the invention, the "phylogenetic profile" method algorithm comprises (a) obtaining data, comprising a list of proteins from at least two genomes; (b) comparing the list of proteins to form a protein phylogenetic profile for each protein, wherein the protein phylogenetic profile indicates the presence or absence of a

protein belonging to a particular protein family in each of the at least two genomes based on homology of the proteins; and (c) grouping the list of proteins based on similar profiles, wherein proteins with similar profiles are indicated to have a functional relationship. The phylogenetic profile can be in the form of a vector, matrix or phylogenetic tree. The "phylogenetic profile" method can further comprise determining the significance of homology between the proteins by computing a probability (*p*) value threshold. The probability can be set with respect to the value 1/NM, based on the total number of sequence comparisons that are to be performed, wherein *N* is the number of proteins in the first organism's genome and *M* in all other genomes. The presence or absence of a protein belonging to a particular protein family in each of the at least two genomes can be determined by calculating an evolutionary distance. The evolutionary distance can be calculated by: (a) aligning two sequences from the list of proteins; (b) determining an evolution probability process by constructing a conditional probability matrix: p(aa→aa'), where aa and aa' are any amino acids, said conditional probability matrix being constructed by converting an amino acid substitution matrix from a log odds matrix to said conditional probability matrix; (c) accounting for an observed alignment of the constructed conditional probability matrix by taking the product of the conditional probabilities for each aligned pair during the alignment of the two sequences, represented by $P(p)=\prod_n p(aa_n \rightarrow aa'_n)$; and, (d) determining an evolutionary distance α from powers equation $p'=p^{\alpha}(aa\rightarrow aa')$, maximizing for P. The conditional probability matrix can be defined by a Markov process with substitution rates, over a fixed time interval. The conversion from an amino acid substitution matrix to a conditional probability matrix can be represented by:

$$P_B(i \rightarrow j) = p(j)2\wedge\frac{\text{BLOSUM62}_{ij}}{2},$$

where BLOSUM62 is an amino acid substitution matrix, and P(i→j) is the probability that amino acid *i* is replaced by amino acid *j* through point mutations according to BLOSUM62 scores. In one aspect, the *Pj*'s are the abundances of amino acid *j* and are computed by solving a plurality of linear equations given by the normalization condition that:

$$\sum_i P_B(i \rightarrow j) = 1.$$

In alternative aspects of the methods of the invention, the "physiologic linkage" method algorithm identifies proteins and nucleic acids that participate in a common functional pathway; identifies proteins and nucleic acids that participate in the synthesis of a common structural complex; and, identifies proteins and nucleic acids that participate in a common metabolic pathway.

In one aspect of the invention, the "domain fusion" method algorithm comprises (a) aligning a first primary amino acid sequence of multiple distinct non-homologous polypeptides to second primary amino acid sequence of a plurality of proteins; and, (b) for any alignment found between the first primary amino acid sequences of all of such multiple distinct non-homologous polypeptides and at least one protein of the second primary amino acid sequences, outputting an indication identifying the aligned second primary amino acid sequence as an indication of a functional link between the aligned first and second polypeptide sequences. The aligning can be performed by an algorithm selected from the group consisting of a Smith-Waterman algorithm, Needleman-Wunsch algorithm, a BLAST algorithm, a FASTA algorithm, and a PSI-BLAST algorithm. The multiple distinct non-homologous polypeptides can be obtained by translating a nucleic acid sequence from a genome database. The plurality of proteins can have a known function. At least one of the multiple distinct non-homologous polypeptides can have a known function. At least one of the multiple distinct non-homologous polypeptides can have an unknown function. The alignment can be based on the degree of homology of the multiple distinct non-homologous polypeptides to the plurality of proteins. The "domain fusion" method can comprise determining the significance of the aligned and identified second primary amino acid sequence by computing a probability (p) value threshold. The probability threshold can be set with respect to the value 1/NM, based on the total number of sequence comparisons that are to be performed, wherein N is the number of proteins in a first organism's genome and M in all other genomes. The "domain fusion" method can further comprising filtering excessive functional links between one first primary amino acid sequence of multiple distinct non-homologous polypeptides and an excessive number of other distinct non-homologous polypeptides for any alignment found between the first primary amino acid sequences of the

distinct non-homologous polypeptides and at least one of the second primary amino acid sequences of the plurality of proteins.

The invention provides a computer program product, stored on a computer-readable medium, for identifying a nucleic acid or a polypeptide sequence that may be a target for a drug, the computer program product comprising instructions for causing a computer system to be capable of: (a) inputting a first nucleic acid or a polypeptide sequence that is known to be a drug target; (b) accessing at least one algorithm capable analyzing a functional relationship between nucleic acid or polypeptide sequences selected from the group consisting of a "domain fusion" method, a "phylogenetic profile" method and a "physiologic linkage" method; and (c) comparing the first nucleic acid or the polypeptide drug target sequence to a plurality of sequences using at least one of the algorithms set forth in step (b) to identify a second sequence that has a functional relationship to the first sequence and generating an output identifying a nucleic acid or a polypeptide sequence that may be a target for a drug .

The invention provides a computer program product, stored on a computer-readable medium, for identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism, the computer program product comprising instructions for causing a computer system to be capable of: (a) providing a first nucleic acid or a polypeptide sequence that is known to be essential for the growth or viability of an organism; (b) accessing at least one algorithm capable analyzing a functional relationship between nucleic acid or polypeptide sequences selected from the group consisting of a "domain fusion" method, a "phylogenetic profile" method and a "physiologic linkage" method; and, (c) comparing the first nucleic acid or the polypeptide sequence to a plurality of sequences using at least one of the algorithms set forth in step (b) to identify a second sequence that has a functional relationship to the first sequence and generating an output identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism.

The invention provides a computer system, comprising: (a) a processor; and, a computer program product of the invention.

All publications, patents, patent applications, GenBank sequences and ATCC deposits, cited herein are hereby expressly incorporated by reference for all purposes.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 is an example of functional linkages predicted between InhA (Rv 1484) and other TB genes.

Figure 2 is an example of predicted functional linkages between embB (Rv 3795), which is a target of the drug ethambutol, and other TB genes using the phylogenetic profile method.

Figure 3 is an example of predicted functional linkages between five TB genes having homology to penicillin binding proteins and other TB genes.

Figure shows that gcpE (Rv 2868C) is predicted to be functional linked to cell wall metabolism.

Figure 5 shows predicted functional linkages of htrA (Rv 1223C) with other TB genes.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

The present invention provides novel methods for identifying the relationships between and the function of nucleic acid and polypeptide sequences. The methods of the invention identify novel genes and polypeptides on the basis of their functional linkage to other proteins whose biological function or processes is known or inferred by homology.

The genes and polypeptides identified by the methods of the invention can be used in screening methods for the identification of compositions which, by binding or otherwise interacting with the gene or polypeptide, are capable of modifying the physiology and growth of an organism. The compositions identified by these screening methods are useful as drugs and pharmaceuticals. Thus, genes and polypeptides identified by the methods

of the invention, including the genes and polypeptides identified herein, can be used as potential drug targets.

One aspect of the invention provides methods for identifying the function of genes and polypeptides from *Mycobacterium tuberculosis* (MTB or TB). Based on this new functional determination, these genes and polypeptides can be used to screen for compositions capable of modifying the physiology and growth of *Mycobacterium tuberculosis* (TB). Thus, genes and polypeptides identified by the methods of the invention, including the genes and polypeptides identified herein, can be used as targets in screening protocols and can be useful as potential drug targets.

The function of the TB genes and polypeptides of the present invention were identified using the methods of the invention; i.e., they were identified on the basis of their functional linkage to other proteins whose biological function or processes were known by experiment or inferred by homology. TB genes and polypeptides that are functionally linked to genes known to be involved in pathogenesis or organisms survival are potential drug targets. Genes or polypeptides associated with TB pathogenesis, survival or that are important or unique to TB biochemical pathways are potential drug targets. TB genes and polypeptides that have no homologues identified in humans are potential drug targets. The function of many of the TB genes and polypeptides identified is based on the genes or polypeptides with which they are functionally linked.

TB genes whose function was identified using the methods of the invention are effectively targeted by a drug (i.e., they can act as *bona fide* drug targets) provides proof of principle that the invention's methods for identifying functionally linked genes can identify TB genes and polypeptides that are drug targets. Further confirmation that the genes identified by the methods of the invention include *bona fide* drug targets can be supported by the fact that genes already known to be targets for drugs have been independently identified, or "re-discovered," by the invention's methods.

The novel TB genes described herein are identified as being functionally related or linked to other genes, including other TB genes, such as a known TB drug target (*e.g.*, InhA polypeptide, which is a target of isoniazid). These functional linkages are established using mathematical algorithms. The assignment or inference of a function to TB genes and polypeptides based on their linkage or relatedness to other genes and polypeptides

is described in U.S. provisional application serial no. 60/165,086. Potential TB drug targets are identified by several methods discussed herein and in further detail in U.S. provisional application serial no. 60/134,092. Through the use of these methods, TB genes and polypeptides have been identified as potential drug targets and are illustrated on Tables 1 and 2, and Figures 1 to 5. The nucleotide and amino acid sequences of these potential drug targets are illustrated on Tables 3 and 4, respectively (see below).

The phrase "functional link," "functionally related" and grammatical variations thereof, when used in reference to genes or polypeptides, means that the genes or polypeptides are predicted to be linked or related. A particular example of functionally related or linked proteins is where two proteins participate in a biochemical or metabolic pathway (*e.g.*, malate dehydrogenase and fumarase, which are both present in the TCA cycle). Thus, although functionally linked or related proteins may not have sequence homology to each other, they are linked by virtue of their participation in the same biochemical pathway. Other examples of linked or related polypeptides are where two polypeptides are part of a protein complex, physically interact, or act upon each another.

The "domain fusion" or "Rosetta Stone" method searches protein sequences across all known genomes and identifies proteins that are separate in one organism but joined as intramolecular domains into one larger protein in another organism. Such proteins that are separate in some organisms but joined in others often carry out related or sequential functions and are therefore functionally linked.

The phylogenetic profile method compares protein sequences across all known genomes and analyzes the pattern of inheritance of each protein across the different organisms. Proteins that have similar patterns of inheritance, either acquired or lost as a part of a group of proteins through evolution, are functionally linked. The gene proximity method identifies genes that remain physically close or "clustered" throughout evolution and are therefore functionally linked.

A particular example of the identification of a potential TB drug target would be to identify a TB gene or polypeptide functionally linked to a known drug target. Anti-TB drugs include isoniazid, rifampicin, ethambutol, streptomycin, pyrazxinamide, and thiacetazone. For isoniazid, this drug is believed to act through enoyl-acyl reductase InhA, resulting in mycolic acid biosynthesis inhibition. Thus, TB genes or polypeptides

functionally linked to enoyl-acyl reductase InhA are potential drug targets; see Figure 1, which shows an analysis of InhA, the target for isoniazid, the most widely used anti-tuberculosis drug, and functional linkages to a set of genes mostly known or hypothesized to be involved in cell wall-related processes and lipid and polyketide metabolism. Particular examples of the identification of several TB genes and polypeptides that are functionally related to the target of these anti-TB drugs is shown in Figures 1 to 5.

"Domain Fusion" or "Rosetta Stone" Method

The "domain fusion" or "Rosetta Stone" method compares protein sequences across known nucleic acid databases (e.g., known genomes) to identify genes and proteins that are separate entities in one organism but are joined into one larger multidomain protein in another organism. In such cases, the two separate proteins often carry out related or sequential functions or form part of a larger protein complex. Therefore, the general function of one component (*e.g.*, one or more of the unknown proteins) can be inferred from the known function of the other component. In addition, merely identifying links between proteins using the method described herein provides valuable information (e.g., usefulness as a target for an antibacterial drug), regardless of whether the function of one or more of the proteins used to form the link(s) is known. Because the two components do not have similar amino acid sequence the function of one could not be inferred from the other on the basis of sequence similarity alone.

The methods for identifying drug targets (e.g., TB drug targets) described herein (*e.g.*, the "Rosetta Stone Method") are based on the idea that proteins that participate in a common structural complex, metabolic pathway, biological process or with closely related physiological functions, are functionally linked. In addition, these methods also are capable of identifying proteins that interact physically with one another. Functionally linked proteins in one organism can often be found fused into a single polypeptide chain in a different organism. Similarly, fused proteins in one organism can be found as individual proteins in other organisms. For example, in a first organism one might identify two un-linked proteins "A" and "B" with unknown function. In another organism, one may find a single protein "AB" with a part that resembles "A" and a part that resembles "B". Protein AB allows one to predict that "A" and "B" are functionally related.

The functional activity of each distinct protein in the "Rosetta Stone" method need not be known prior to performing the method (*i.e.*, the function of A, B, or AB need not be known). Using the "Rosetta Stone" method to compare and analyze several unknown protein sequences can provide information regarding relationships of each protein absent knowledge about the functional activity of the initially analyzed proteins themselves. For example, the information (*i.e.*, the links) can provide information that the proteins are part of a common pathway, function in a related process or physically interact. Such information need not be based on the biological function of the individual proteins.

These methods can provide information regarding links between previously un-linked proteins that function, for example, in a concerted process. A marker, for example, for a particular disease state is identified by the presence or absence of a protein (*e.g.*, Her2/neu in breast cancer detection). Links (*i.e.*, information) identified by the method, which link proteins "B" and "C" to such a marker suggest that proteins "B" and "C" are related by function, physical interaction or part of a common biological pathway with the marker. Such information is useful in designing screening methods and identifying drug targets (e.g., TB drug targets), making diagnostics, and designing therapeutics.

In one approach, the "Rosetta Stone" method is performed by sequence comparison that searches for incomplete "triangle relationships" between, for example, three proteins, *i.e.*, for two proteins A' and B' that are different from one another but similar in sequence to another protein AB. Completing the triangle relationship provides useful information regarding the proteins' biological function(s), functional interaction, pathway relationships or physical relationships with other proteins in the "triangle."

Either nucleotide sequences or amino acid sequences can be used in the methods for identifying functionally related or linked genes or polypeptides. Where a nucleic sequence is to be used it can be first translated from a nucleic acid sequence to amino acid sequence. Such translation may be performed in all frames if the coding sequence is not known. Programs that can translate a nucleic acid sequence are known in the art. In addition, for simplicity, the description of this method discusses the use of a "pair" of proteins in the determination of a "Rosetta Stone" protein, more than 2 may be used (*e.g.*, 3, 4, 5, 10, 100 or more proteins). Accordingly, one can analyze chains of linked proteins, such as "A" linked by a Rosetta Stone protein to "B" linked by a Rosetta Stone protein to "C", etc.

By this method, groups of functionally related proteins can be found and their function identified.

A method can start with identifying the primary amino acid sequence for a plurality of proteins whose functional relationship is to be determined (*e.g.*, protein A' and protein B'). A number of source databases are available, as described above, that contain either a nucleic acid sequence and/or a deduced amino acid sequence for use with the first step. The plurality of sequences (the "probe sequences") are then used to search a sequence database, *e.g.*, GenBank (NCBI, NLM, NIH), PFAM (a large collection of multiple sequence alignments and hidden Markov models covering many common protein domains; Washington University, St. Louis MO) or ProDom (a database based on recursive PSI-BLAST searches and designed as a tool to help analyze domain arrangements of proteins and protein families, see, e.g., Corpet (1999) Nucleic Acids Res. 27:263-267), either simultaneously or individually. Every protein in the sequence database is examined for its ability to act as a "Rosetta Stone" protein (*i.e.*, a single protein containing polypeptide sequences or domains from both protein A' and protein B'). A number of different methods of performing such sequence searches are known in the art. Such sequence alignment methods include, for example, BLAST (see, e.g., Altschul (1990) J. Mol. Biol. 215: 403-410), BLITZ (MPsrch) (see, e.g., Brenner (1995) Trends Genet. 11:330-331; and infra), and FASTA (see, e.g., Pearson (1988) Proc. Natl. Acad. Sci. USA 85(8):2444-2448; and infra). The probe sequence can be any length (*e.g.*, about 50 amino acid residues to about 1000 amino acid residues).

Probe sequences (e.g., polypeptide sequences or domains) found in a single protein (*e.g.*, an "AB" multidomain protein) are defined as being "linked" by that protein. Where the probe sequences are used individually to search the sequence database, one can mask those segments having homology to the first probe sequence found in the proteins of the sequence database prior to searching with the subsequent probe sequence. In this way, one eliminates any potential overlapping sequences between the two or more probe sequences.

The linked proteins can then be further compared for similarity with one another by amino acid sequence comparison. Where the sequences are identical or have high homology, such a finding can be indicative of the formation of homo-dimers, -trimers, etc.

Typically, "Rosetta Stone"-linked proteins are only kept when the linked proteins show no homology to one another (*e.g.*, hetero-dimers, trimers, etc.).

In another method for identifying functional linkages, a potential fusion protein lacking any functional information that is suspected of having two or more domains (*e.g.*, a potential "Rosetta Stone" protein) may be used to search for related proteins. In this method, the primary amino acid of the fusion protein is determined and used as a probe sequence. This probe sequence is used to search a sequence database (*e.g.*, GenBank, PFAM or ProDom). Every protein in the sequence database is examined for homology to the potential fusion protein (*i.e.*, multiple proteins containing polypeptide sequences or domains from the potential fusion protein). A number of different methods of performing such sequence searches are known in the art, *e.g.*, BLAST, BLITZ (Biocomputing Research Unit, University of Edinburgh, Scotland, the "MPsrch program" performs comparisons of protein sequences against the Swiss-Prot protein sequence database using the Smith and Waterman best local similarity algorithm), and FASTA.

Probe sequences found in more than one protein (*e.g.*, A' and B' proteins) are defined as being "linked" so long as at least one protein per domain containing that domain but not the other is also identified. In other words, at least one protein or domain of the plurality of proteins must also be found alone in the sequence database. This verifies that the protein or domain is not an integral part of a first protein but rather a second independent protein having its own functional characteristics.

Statistical methods can be used to judge the significance of possible matches. The statistical significance of an alignment score is described by the probability, P, of obtaining a higher score when the sequences are shuffled. One way to compute a P value threshold is to first consider the total number of sequence comparisons that are to be performed. For example, if there are N proteins in *E. coli* and M in all other genomes this number is $N \times M$. If a comparison of this number of random sequence would result in one pair to yield a P value of $1/NM$ by chance this then is set as the threshold.

This method provides information regarding which proteins are functionally related (*e.g.*, related biological functions common structural complexes, metabolic pathways or biological process) a subset of which physically interact in an organism.

Alignment Algorithms

To align sequences, a number of different procedures can be used that produce a good match between the corresponding residues in the sequences. Typically, the Smith-Waterman (Smith (1981) Adv. Appl. Math. 2:482) or Needleman-Wunsch algorithm (Needleman (1970) J. Mol. Biol. 48:443) algorithm, are used, however, other, faster procedures such as BLAST, FASTA, PSI-BLAST (a version of Blast for finding protein families), or others known in the art (see infra discussion), can be used.

Filtering Methods

The Rosetta Stone Method provides at least two pieces of information. First the method provides information regarding which proteins are functionally related. Second the method provides information regarding which proteins are physically related. Each of these two pieces of information has different sources of error and prediction. The first type of error is introduced by protein sequences that occur in many different proteins and paired with many other protein sequences. The second type of error is introduced due to there often being multiple copies of similar proteins, called paralogs, in a single organism. In general, the "Rosetta Stone" method predicts functionally related proteins well, with no filtering of results required. However, it is possible to filter the error associated with either the first or second type of information.

The invention recognizes that a few domains are linked to an excessive number of other domains by a "Rosetta Stone" protein. For example, 95% of the domains are linked to fewer than 25 other domains. However, some domains, *e.g.*, the Src Homology 3 (SH3) domain or ATP-binding cassette (ABC domains), link to more than a hundred other domains. These links were filtered by removing all links generated involving these 5% of domains (*i.e.*, the domains linked to more than 25 other domains). For example, in *E. coli*, without filtering, 3531 links were identified using the domain-based analysis, but after filtering only 749 links were identified. This method improved prediction of functionally related proteins by 28% and physically related proteins by 47%. Accordingly, there are a number of ways to filter the results to improve the significance of the functional links. As described above, as the number of functional links increases there is an increased higher chance of finding a "Rosetta Stone" protein. By reducing the excessively linked proteins one

reduces the chance number of "Rosetta Stone" proteins thereby increasing the significance of a functional link.

Error introduced by multiple paralogs of linked proteins should have little effect on functional prediction, as paralogs usually have very similar function, but will affect the reliability of prediction of protein-protein interactions. This estimate is calculated for each linked protein pair, and can be estimated roughly as:

$$\text{Fractional Error} = 1 - \frac{\sqrt{N}}{N},$$

where N is the number of paralogous protein pairs, (*e.g.*, A linked to B, A' linked to B', A linked to B', and A' linked to B, in the case that A and A' are paralogs, as are B and B', and the linking proteins is AB as above).

The error can also be estimated as $1-T$, where T is the mean percent of potential true positives calculated for all domain pairs in an organism. For each domain pair linked by a Rosetta Stone protein, there are n proteins with the first domain but not the second, and m proteins with the second domain but not the first. The percent of true positives T is therefore estimated as the smaller of n or m divided by n times m. As this error T can be calculated for each set of linked domains, it can describe the confidence in any particular predicted interaction.

In addition, the error in functional links can be caused by small conserved regions or repeated common amino acid sequences being repeatedly identified in a "Rosetta Stone" protein by a plurality of distinct non-homologous polypeptides. To reduce this error the percent of identity between the "Rosetta Stone" and the distinct non-homologous polypeptide can be measured. Alignment percentages of about 50% to about 90%, or, alternatively, about 75%, between the "Rosetta Stone" and the distinct polypeptide are indicative of links that are not subject to the small peptide sequence.

Phylogenetic Pathway Method

The "phylogenetic profile" method compares protein sequences across all known genomes and analyzes the pattern of inheritance of each protein across the different organisms. In its simplest form, each protein is simply characterized by its presence or absence in each organism. For example, if there are 16 known genomes, then each protein may be assigned a 16-bit code or phylogenetic profile. Since proteins that function together

(*e.g.*, in the same metabolic pathway or as part of a larger functional or structural complex) evolve in a correlated fashion, they should have the same or similar patterns of inheritance, and therefore similar phylogenetic profiles. Therefore, the function of one protein may be inferred from the function of another protein, which has a similar profile, if its function is known. As with the Rosetta Stone method, the function of one protein is inferred from the function of another protein which is dissimilar in sequence. Furthermore, the predicted link between the proteins has utility in developing, for example, drug targets, diagnostics and therapeutics.

The phylogenetic profile method can be implemented in a binary code (*i.e.*, describing the presence or absence of a given protein in an organism) or a continuous code that describes how similar the related sequences are in the different genomes. In addition, grouping of similar protein profiles may be made wherein similar profiles are indicative of functionally related proteins. Furthermore, the requirements for similarity can be modified depending upon particular criteria by varying the difference in similar bit requirements. For example, criteria requiring that the degree of similarity in the profile include all 16 bits being identical can be set, but may be modified so that similarity in 15 bits of the 16 bits would indicate relatedness of the protein profiles as well. Statistical methods can be used to determine how similar two patterns must be in order to be related.

The phylogenetic profile method is applicable to any genome including, e.g., viral, bacterial, archaeal or eukaryotic. The method of phylogenetic profile grouping provides the prediction of function for a previously uncharacterized protein(s). The method also allows prediction of new functional roles for characterized proteins based upon functional linkages. It also provides potential informative connections (*i.e.*, links) between uncharacterized proteins.

To represent the subset of organisms that contain a homolog a phylogenetic profile is constructed for each protein. The simplest manner to represent a protein's phylogenetic history is via a binary phylogenetic profile for each protein. This profile is a string with N entries, each one bit, where N corresponds to the number of genomes. The number of genomes can be any number of two or more (*e.g.*, 2, 3, 4, 5, 10, 100, to 1000 or more). The presence of a homolog to a given protein in the n^{th} genome is indicated with an entry of unity at the n^{th} position (*e.g.*, in a binary system an entry of 1). If no homolog is

found the entry is zero. Proteins are clustered according to the similarity of their phylogenetic profiles. Similar profiles show a correlated pattern of inheritance, and by implication, functional linkage. The method predicts that the functions of uncharacterized proteins are likely to be similar to characterized proteins within a cluster.

In order to decide whether a genome contains a protein related to another particular protein, the query amino acid sequence is aligned with each of the proteins from the genome(s) in question using known alignment algorithm (see above). To determine the statistical significance of any alignment score, the probability, p, of obtaining a higher score when the sequences are shuffled is described. One way to compute a p value threshold is to first consider the total number of sequence comparisons that are being aligned. If there are N proteins in a first organism's genome and M in all other genomes this number is $N \times M$. If this number were compared to random sequences it would be expected that one pair would yield a p value of $\frac{1}{NM}$. This value can be set as a threshold. Other thresholds may be used and will be recognized by those of skill in the art.

A non-binary phylogenetic profile can be used. In this method, the phylogenetic profile is a string of N entries where the n^{th} entry represents the evolutionary distance of the query protein to the homolog in the n^{th} genome. To define an evolutionary distance between two sequences an alignment between two sequences is performed. Such alignments can be carried out by any number of algorithms known in the art (for examples, see those described above). The evolution is represented by a Markov process with substitution rates, over a fixed interval of time, given by a conditional probability matrix:

$$p(aa \rightarrow aa')$$

where aa and aa' are any amino acids. One way to construct such a matrix is to convert the BLOSUM62 amino acid substitutions matrix (or any other amino acid substitution matrix, *e.g.*, PAM100, PAM250) from a log odds matrix to a conditional probability (or transition) matrix:

$$P_B(i \rightarrow j) = p(j)2^{\frac{\text{BLOSUM62}_{ij}}{2}} \qquad (1)$$

$P(i \rightarrow j)$ is the probability that amino acid i will be replaced by amino acid j through point mutations according to the BLOSUM62 scores. The p_j's are the abundances of amino

acid j and are computed by solving the 20 linear equations given by the normalization conditions that:

$$\sum_i P_B(i \rightarrow j) = 1 \qquad . \qquad (2)$$

The probability of this process is computed to account for the observed alignment by taking the product of the conditional probabilities for each aligned pair:

$$P(p) = \prod_n p(aa_n \rightarrow aa'_n) \qquad . \qquad (3)$$

A family of evolutionary models is then tested by taking powers of the conditional probability matrix: $p' = p^{\alpha}(aa \rightarrow aa')$. The power α that maximized P is defined to be the evolutionary distance.

Many other schemes may be imagined to deduce the evolutionary distance between two sequences. For example, one might simply count the number of positions in the sequence where the two proteins have adapted different amino acids.

Although the phylogenetic history of an organism can be presented as a vector (as described above), the phylogenetic profiles need not be vectors, but may be represented by matrices. This matrix includes all the pair wise distances between a group of homologous protein, each one from a different organism. Similarly, phylogenetic profiles could be represented as evolutionary trees of homologous proteins. Functional proteins could then be clustered or grouped by matching similar trees, rather than vectors or matrices.

In order to predict function, different proteins are grouped or clustered according to the similarity of their phylogenetic profiles. Similar profiles indicate a correlated pattern of inheritance, and by implication, functional linkage.

Grouping or clustering may be accomplished in many ways. The simplest is to compute the Euclidean distance between two profiles. Another method is to compute a correlation coefficient to quantify the similarity between two profiles. All profiles within a specified distance of the query profile are considered to be a cluster or group.

Typically a genome database will be used as a source of sequence information. Where the genome database contains only the nucleic acid sequence that sequence is translated to an amino acid sequence in frame (if known) or in all frames if unknown. Direct comparison of the nucleic acid sequences of two or more organisms may be feasible but will likely be more difficult due to the degeneracy of the genetic code.

Programs capable of translating a nucleic acid sequence are known in the art or easily programmed by those of skill in the art to recognize a codon sequence for each amino acid.

The phylogenetic profile provides an indication of those proteins in each of the at least two organisms that share some degree of homology. Such a comparison can be done by any number of alignment algorithms known in the art or easily developed by one skilled in the art (see, for example, those listed above, *e.g.*, BLAST, FASTA etc.) In addition, thresholds can be set regarding a required degree of homology. Each protein is then grouped at 224 with related proteins that share a similar phylogenetic profile using grouping algorithms.

"Functionally-, Structurally- or Metabolically- Linked" Method

The "physiologic linkage" method is a computational method that detects (i.e., identifies) proteins, and the genes that encode them, that participate in a common functional pathway (e.g., cell motility or cell division), that participate in the synthesis of the same or a similar structural complex (e.g., a cell wall) or participate in the same or similar metabolic pathway (e.g., glycolysis, lipid synthesis, and the like). Proteins within these common functional pathway groups are examples of "functionally linked" proteins. Having a common functional "goal" they evolve in a correlated fashion. Thus, "homologs" in different organisms can be comparatively identified. While these detection methods are very effective in identifying functional homologues in the same subset of organisms, functional linkages can be made between widely genetically disparate organisms.

In one aspect, metabolic pathways are defined as links between proteins that operate in the same metabolic pathway that can be identified by sequence identity searching, e.g., by performing a BLAST search to find top-scoring polypeptides with high similarity (BLAST alignment E-value < 10^{-20}) to polypeptides identified in a known pathway. For example, *M. tuberculosis* proteins were so analyzed against *E. coli* proteins; MTB proteins whose *E. coli* homologs (i.e., having high similarity by BLAST alignment) act adjacently in metabolic pathways as defined in the EcoCyc database (see, e.g., Karp (1998) Nucleic Acids Res. 26:50-53) were identified.

In another example, flagellar proteins are found in bacteria that possess flagella but not in other organisms. Accordingly, if two proteins have homologs in the same subset of fully sequenced organisms, they are likely to be functionally linked. The methods

of the invention use this concept to systematically map links between all the proteins coded by a genome.

Typically, functionally linked proteins have no amino acid sequence similarity with each other and, therefore, cannot be linked by conventional sequence alignment techniques. Accordingly, the methods of the invention identify drug targets that could not be identified using conventional sequence comparison (i.e., sequence homology or sequence identity) techniques.

Prediction of functionally linked proteins by the "phylogenetic method" can also be used in conjunction with the "domain fusion" or "Rosetta Stone" method and also can be filtered by other methods that predict functionally linked proteins, such as the protein phylogenetic profile method or the analysis of correlated mRNA expression patterns. It was found that filtering by these two methods for the Rosetta Stone prediction for *S. cerevisiae*, that proteins predicted to be functionally linked by two or more of these three methods were as likely to be functionally related as proteins that were observed to physically interact by experimental techniques like yeast 2-hybrid methods or co-immunoprecipitation methods.

For example, a combination of these methods of prediction can be used to establish links between proteins of closely related function. The methods of the invention (*i.e.*, the "Rosetta Stone" method and the "phylogenetic profile" method) can be combined with one another or with other protein prediction methods known in the art; see, for example, Eisen (1998) "Cluster analysis and display of genome-wide expression partners," *Proc. Natl. Acad. Sci. USA*, 95:14863-14868.

The various techniques, methods, and variations thereof described can be implemented in part or in whole using computer-based systems and methods. Additionally, computer-based systems and methods can be used to augment or enhance the functionality described above, increase the speed at which the functions can be performed, and provide additional features and aspects as a part of or in addition to those of the invention described elsewhere in this document. Various computer-based systems, methods, and implementations in accordance with this technology are described herein.

Proteins linked to current drug targets

The invention also provides a novel method for identifying a polypeptide, or the nucleic acid sequence that encodes it, that is a target for a drug. The method analyzes the

functional relationship between at least two sequences, wherein at least one of the sequences is a known target of a drug or encodes a polypeptide drug target. The method comprises identifying proteins, and the genes that encode them, that are functionally linked to the targets of known drugs. The functional linkage is determined by using the "domain fusion" method, the "phylogenetic profile" method or the "physiologic linkage" method, or a combination thereof, as described herein.

Thus, this aspect of the invention provides methods identifying drug targets from among all or a subset of genes in a genome using computationally-determined functional linkages. In one implementation of the method, functional linkages are calculated using the "domain fusion" method, the "phylogenetic profile" method or the "physiologic linkage" method, or a combination thereof, between all "query genome genes." Next, each set of genes predicted to be functionally linked to either a known drug target or to a sequence homolog or ortholog (defined below) to a known drug target are examined. These proteins (and the nucleic acids that encode them) are functionally linked to known drug targets; thus, they are operating in the same pathways or systems targeted by the known drug. Accordingly, the methods of the invention have identified them as drug targets.

This method is particularly effective for identifying drug targets in pathogens, such as microorganisms, e.g., bacteria, viruses and the like. This method allows for the identification of novel drug targets that cannot be identified by other techniques, such as traditional sequence homology or sequence identity comparison techniques. Several known drug targets in *M. tuberculosis* were used with the methods of the invention to use functional linkages to identify potential new drug targets in the same pathways as the known drug targets.

There are very few drugs that are effective for anti-tuberculosis therapy, since the complex lipid-rich mycobacterial cell wall is impermeable to many antibacterial agents. Additionally, single- and multi-drug resistance is rapidly emerging against these drugs. To address this issue, the methods of the invention were used to identify *Mycobacterium tuberculosis* (MTB or TB) proteins that are functionally linked to the targets of known drugs. Inhibiting these proteins should have the same effect on the organism as the drug, since the same processes or pathways would be disrupted. Targeting multiple components of a given biochemical pathway would also diminish the opportunity for the development of resistance

because various related proteins would have to mutate against inhibitors while preserving the overall functionality of the pathway.

A list of targets of essential anti-TB drugs (World Health Organization, Geneva, Switzerland) was compiled. The anti-TB drugs included isoniazid, rifampicin, ethambutol, streptomycin, pyrazinamide and thiacetazone. Although not enough is known about the molecular basis of action of the latter two, the functional linkages of the known drug targets was examined.

Isoniazid. This is one of the most widely used of all anti-tuberculosis drugs. It is believed that the compound is activated by the catalase-peroxidase KatG. Once activated, it then attaches to a nicotinamide adenine dinucleotide bound to the enoyl-acyl carrier protein reductase InhA, resulting in the inhibition of mycolic acid biosynthesis Rozwarski (1998) Science 279:98-102.

Using the "phylogenetic profile, the inhA gene was "linked," or functionally associated with, to two polyketide synthases, pks1 and pks6 (Figure 1), both of which contain acyl carrier protein motifs. The polyketide synthase pks6 is in turn known from established metabolic pathways to be linked to fatty acid biosynthesis gene accD3. Further, pks6 is linked to fadD28 and to the operon containing the genes ppsA-E, all recently reported to be crucial for bacterial replication in host lungs (see, e.g., Cox (1999) Nature 402:79-83).

The inhA gene was also linked to an operon encoding two putative oxidoreductases and a gene of entirely unknown function. The inhA gene was further linked to a second operon that includes pepR and gpsI. PepR is a protease whose *Bacillus subtilis* homolog is adjacent to the genes coding for enzymes that synthesize diaminopimelate, a component of the cell wall incorporated by the murE gene product and diaminopicolinate (see, e.g., Chen (1993) J. Biol. Chem. 268:9448-9465). PepR is an ortholog of an essential yeast gene and is likely to be essential for MTB (see below). GpsI is a putative multifunctional enzyme involved in guanosine pentaphosphate synthesis and polyribonucleotide nucleotidyltransfer. The high reliability of the predicted functional link between gpsI and pepR and the absence of eukaryotic homologs suggests that gpsI could be a promising target for drug design.

Rifampicin. This compound, along with the related rifabutin and KRM-1648 are believed to act by directly targeting the RNA polymerase β-subunit (rpoB) given that

96% of resistant isolates were found to have mutations of various types in a limited region of the rpoB gene (see, e.g., Yang (1998) J. Antimicrob. Chemother. 42:621-628).

Using the methods of the invention, as expected, functional linkages were found to another RNA polymerase subunit, rpoC, as well as to various tRNA synthases and ribosomal proteins. However, no functional links to uncharacterized proteins were found.

Ethambutol. This drug is effective against tuberculosis when used in combination with isoniazid. It is believed that the drug interacts with the EmbB protein, a probable arabinosyl-transferase, inhibiting the biosynthesis of arabinan, a component of cell-envelope lipids. As with rifampicin, the evidence for this interaction is indirect, since mutations in the embB gene are responsible for ethambutol resistance (see, e.g., Lety (1997) Antimicrob. Agents Chemother. 41:2629-2633).

The "gene proximity" method correctly clusters embB with embA (Rv3794). This cluster is linked to a set of mostly uncharacterized genes by the "phylogenetic profile" method; see Figure 2, which shows an analysis of EmbB, the target for the anti-tuberculosis drug Ethambutol, and shows functional linkages to genes mostly of unknown function but with some indications of localization at the bacterial membrane.

Two of the uncharacterized genes, Rv1706c and Rv1800, belong to the abundant PE/PPE family of proteins hypothesized to be a source of antigenic variation with the potential ability to interfere with immune responses by inhibiting antigen processing (see, e.g., Cole (1998) Nature 393, 537-544). A third uncharacterized gene, Rv1967 belongs to the one of the four copies of the mce operon. This operon consists of eight genes coding for integral membrane proteins and proteins that have N-terminal signal sequences or hydrophobic segments and are believed to be involved in pathogenicity (see, e.g., Cole (1998) supra). Rv0528 codes for a hypothetical membrane protein and Rv2159c corresponds to the murF gene, which participates in the biosynthesis of peptidoglycan precursors.

The majority of the "links," or functionally associated sequences, involved proteins associated with processes related to the bacterial cell wall (with the possible exception of atsA and the putative choline dehydrogenase Rv1279, whose relationship to these processes is not immediately obvious). The proteins of unknown function are therefore also expected to play some role in these processes and are thus of interest as potential drug targets.

Streptomycin. This drug acts by binding to the 16S rRNA and inhibits protein synthesis. Resistance to this compound emerges from mutations in the corresponding gene (rrs), as well as in the gene encoding for the ribosomal protein S12 (rpsL). Disruptions to RpsL effect streptomycin resistance by altering the higher order structure of 16S rRNA (see, e.g., Sreevatsan (1996) Antimicrob. Agents Chemother. 40:1024-1026).

Although streptomycin doesn't directly target RpsL, the functional links generated for this protein was examined, as any target whose inhibition will ultimately disrupt bacterial protein synthesis is likely to be an effective antigrowth/ anti-microbial target. As with the rifampicin target, the only functional linkages found for this protein were the expected protein synthesis-related proteins, including large ribosomal subunit proteins L2, L5, L11, and L14; small ribosomal subunit proteins S4, S5, S7, S8, and S11; elongation factors fusA and Ef-Tu; the chaperones GroEL, clpB and ftsH; and the Clp protease subunits clpC and clpX.

Proteins linked to cell-wall related proteins

The invention also provides a novel method for identifying a nucleic acid or a polypeptide sequence in an organism that is linked to a cell-wall related protein. The method analyzes the functional relationship between at least two sequences, wherein at least one of the sequences is a cell-wall related protein, or, the sequence is a nucleic acid sequence that encodes a cell-wall related protein. The method comprises identifying proteins, and the genes that encode them, that are functionally linked to a cell-wall related protein. The functional linkage is determined by using the "domain fusion" method, the "phylogenetic profile" method or the "physiologic linkage" method, or a combination thereof, as described herein.

Approximately eleven *M. tuberculosis* proteins are indicated by sequence homology to be penicillin-binding proteins, thought to synthesize peptidoglycan in the course of cell elongation and cell wall metabolism (see, e.g., Broome-Smith (1985) Eur. J. Biochem. 147:437-446). Using the methods of the invention, the functional linkages found for these proteins map out many of the known cell wall synthetic enzymes and reveal more than 10 proteins of unknown function that may also participate in cell wall metabolism. Figure 3 shows an analysis of five of the approximately eleven MTB proteins presumed to bind penicillin to reveal functional linkages to various potential operons consisting of genes

involved in various aspects of cell wall metabolism, including cell shape determination and peptidoglycan biosynthesis, as well more than ten genes of unknown function, which we can now associate with cell wall metabolism.

Three of the proteins (pbpA, pbpB, and ponA1) reside in conserved gene clusters, presumably operons. Other genes in the clusters around pbpA and pbpB are also implicated in cell wall metabolism. For example, pbpA resides next to rodA, a membrane-associated protein whose *E. coli* homolog determines cell shape and is required for enzymatic activity of penicillin binding proteins (see, e.g., Matsuzawa (1989) J. Bacteriol. 171:558-560). Likewise, pbpB resides next to six peptidoglycan biosynthesis genes and the two septum and cell wall formation proteins ftsW and ftsZ.

Two additional gene clusters were linked to these penicillin binding proteins by either the "phylogenetic profile" or "Rosetta Stone" pattern methods of the invention. One cluster is composed of the peptidoglycan synthetic protein murB and a putative membrane protein of unknown function that the functional linkages suggest is involved in cell wall metabolism. The second gene cluster contains four genes, three of which are predicted to reside in the cell membrane or envelope. Therefore, the uncharacterized genes in these clusters are likely to be involved in cell wall metabolism, closely related to the function of the penicillin binding proteins and are therefore promising drug targets.

Another gene linked to cell wall metabolism by the computationally-derived linkage methods of the invention is gcpE, see Figure 4, which shows that the uncharacterized gene gcpE, known to be essential for bacterial survival (see, e.g., Baker (1992) FEMS Microbiol. Lett. 73:175-180), is predicted to be involved in cell wall metabolism through its functional links to a putative membrane protein and two murein hydrolase genes, lytB1 and lytB2, involved in cell separation. The genes forming a putative operon with gcpE are proposed as potential drug targets. The functional linkages place gcpE in a conserved gene cluster with two genes of unknown function, one of which encodes a membrane protein. However, the three genes show correlated inheritance with two homologs of lytB, an *E. coli* gene involved in penicillin tolerance (see, e.g, Gustafson (1993) J. Bacteriol. 175:1203-1205) and recently shown to encode a murein hydrolase essential for cell separation (see, e.g., Garcia (1999) Mol. Microbiol. 31:1275-1277). The uncharacterized proteins from this

cluster are therefore expected to participate in processes similar to GcpE and might therefore be promising drug targets.

Proteins linked to potentially novel pathways

The invention also provides a novel method for identifying a polypeptide, or a nucleic acid that encodes it, that is linked to potentially novel biochemical (e.g., biosynthetic, metabolic) pathways. The method analyzes the functional relationship between at least two sequences, wherein at least one of the sequences is associated with a biochemical pathway, such as a pathway in a microorganism that enables the pathogen to evade an immune process. The method comprises identifying proteins, and the genes that encode them, that are functionally linked to the pathway-linked sequences. The functional linkage is determined by using the "domain fusion" method, the "phylogenetic profile" method or the "physiologic linkage" method, or a combination thereof, as described herein.

For example, the htrA gene encodes for a putative heat shock protein homologous to HtrA from *Salmonella typhimurium*, a serine protease that degrades aberrant periplasmic proteins. Mutations in this protein have been linked with reduced viability in host macrophages (see, e.g., Johnson (1991) Mol. Microbiol. 5:401-407). Thus, it was decided to investigate the function of htrA. Using the methods of the invention, results indicated that the htrA protein is part of a process that has not yet been characterized. The gene is predicted with very high reliability to function with the uncharacterized gene Rv1224c, see Figure 5, which shows the involvement of htrA in a potentially novel pathway and the gene encoding the putative heat shock protein HtrA is functionally linked to a set of genes mostly of unknown function, suggesting the existence of a novel pathway. The partially characterized proteins suggest that the pathway relates to membrane-associated processes such as signaling and/or transport. The lack of eukaryotic homologs for most of the genes linked to htrA, suggests that proteins of this pathway could be promising drug targets.

Through its phylogenetic profile, htrA is linked to a group of uncharacterized proteins, including a putative lipid esterase (Rv1900c), an ABC transporter (Rv3783) and the uncharacterized protein Rv1216c, which has weak homology to the laminin B receptor of *Xenopus laevis*, suggesting that it might be a membrane protein. From this analysis, it can be concluded that htrA is part of a novel pathway that involves membrane-associated processes,

such as signaling and/or transport. Because the majority of the proteins linked to htrA have no eukaryotic homologs, and given the importance of htrA in *S. typhimurium* pathogenesis, this pathway represents another potential source of novel targets for anti-tuberculosis drugs.

Proteins linked to essential proteins

The invention also provides a novel method for identifying a polypeptide, or the nucleic acid sequence that encodes it, that is linked to an essential protein (e.g., a protein necessary for the growth of an organism, such as a bacterium). The method analyzes the functional relationship between at least two sequences, wherein at least one of the sequences is linked to an essential protein, or, the sequence is a nucleic acid sequence that itself is essential or encodes a polypeptide linked to an essential protein. The functional linkage is determined by using the "domain fusion" method, the "phylogenetic profile" method or the "physiologic linkage" method, or a combination thereof, as described herein.

For example, the MIPS database (Munich Information Center for Protein Sequences; MIPS provides access through its WWW server to a spectrum of generic databases, including PEDANT, MYGD, MATD, MEST, the PIR-International Protein Sequence Database, the protein family database PROTFAM, the MITOP database, and the all-against-all FASTA database; see, e.g., Mewes (1999) Nucleic Acids Res. 27:44-48) contains a list of 734 genes that are essential for *Saccharomyces cerevisiae* viability (see, e.g., Mewes (1999) supra). A list of *Mycobacterium tuberculosis* genes orthologous to these essential genes was generated. Using the methods of the invention, 60 such genes were found. The products of these genes have a high likelihood of also being essential to the tuberculosis bacterium and therefore could be promising therapeutic targets. Furthermore, since the list of essential genes came from a eukaryote, there is a significant chance that these genes would also be found in the human genome.

Automatic Method to Identify Drug Targets from Functional Linkages

One aspect of the invention provides a computational method to identify potential drug targets among the proteins expressed by a genome. This aspect takes advantage of the functional linkages calculated between genes in a genome using the methods described herein, as well as the detection of sequence homology and the knowledge of a set of lethal or "essential" genes in one or more organisms.

To identify drug targets in a query genome, the sequence homology between all of the genes in that genome and all of the genes in the genome of an organism for which essential genes are known is calculated. For example, as discussed herein, the query genome is Mycobacterium tuberculosis (TB) and the genome with known essentials is the yeast S. cerevisiae. Sequence homology between all TB genes and all yeast genes was calculated using the methods of the invention.

"Equivalent" or "orthologous" genes were also identified by another aspect of the invention that comprises doing a reverse sequence search (e.g., yeast vs. TB) and then choosing pairs of genes that are the symmetric best-scoring sequence search. In one exemplary aspect, MTB orthologs of *Saccharomyces cerevisiae* genes were generated by finding all pairs of genes (TB_i,SC_j) where TB_i was the top hit from a BLAST search of the yeast gene SC_j against the MTB genome, SC_j was the top hit from a BLAST search of the MTB gene TB_i against the *Saccharomyces cerevisiae* genome and both top hits had a BLAST E-value <= $1x10^{-5}$.

For example, a TB gene is an ortholog of a yeast gene if the yeast gene is the best scoring sequence match when yeast is searched with the TB gene, and the TB gene is the best scoring sequence match when TB is searched with the yeast gene. We define these "symmetric" pairs as "orthologs."

After identifying orthologs between the query genome and the genome with known essential genes, a set of query genome genes that are orthologs of known essential genes in the other genome was chosen. These genes were designated the set of "putative essentials". For the purposes of the algorithm of the invention, these query genome genes are assumed to be essential genes, since they are the equivalents of essential genes in another genome. These genes act as "markers" or indicators of essential pathways in the query genome. One could supplement this set with genes already known to be essential in the query organism. Functional linkages (determined by the methods of the invention) between all query genome genes were examined. The query genome genes linked to all of the putative essential genes were examined. This set of genes was designated as the "predicted members of essential pathways." These genes are likely to be involved in important pathways, since the (predicted) pathways have members that are putative essentials. Lastly, the method removes from the set of genes in predicted essential pathways all of those genes

that have sequence homology to eukaryotic genes or proteins. The genes that remain after this filtering step are the predicted drug targets for the query organism.

As a benchmark, this method was applied to the *M. tuberculosis* genome. Of the over 3900 genes in TB, 11 were identified as potential drug targets. Comparing this list of 11 predicted targets to the less than 10 known drug anti-TB drug targets, one gene was a known drug target and one was linked to a known drug target. Accordingly, the algorithm of the invention performed statistically significantly much better than a random choice of genes. A rough estimate of statistical significance suggests that one would expect to see 2 of 10 known drug targets in a sample of 11 out of 3900 genes only 3.8 times out of 10,000 trials (probability of occurring by random chance of 3.8×10^{-4}). Therefore, this embodiment of the method is an entirely computational algorithm drawing on the demonstrated ability of the general methods of the invention to predict functional linkages between genes and to effectively identify drug targets in bacteria. The effectiveness of this method to identify novel drug targets was clearly demonstrated when the algorithm was applied to the *M. tuberculosis* genome.

The specific inhibition of the MTB homologs might be difficult. To address this issue, using the methods of the invention, functional links to the essential genes were searched. Functional links were selected which either do not have homologs in yeast, or the enzymatic activity of their products are known to be absent in human cells. Using the highest confidence data, functional links for 23 of the genes (indicated in **bold** in Table 1) were found.

Table 1. MTB orthologs of essential yeast proteins.

Name[†]	Gene	Comments	Name	Gene	Comments
Rv0005[‡]	gyrB	DNA gyrase subunit B	Rv2101	helZ	probable helicase, Snf2/Rad54 family
Rv0014c	pknB	serine-threonine protein kinase	Rv2110c	prcB	proteasome [beta]-type subunit 2
Rv0032	bioF2	C-terminal similar to B. subtilis BioF	Rv2118c	-	= B2126_C1_165 (83.6%)
Rv0350	dnaK	70 kD heat shock protein, chromosome replication	Rv2438c	-	similar to YHN4_YEAST P38795
Rv0363c	fba	fructose bisphosphate aldolase	**Rv2439c**	proB	glutamate 5-kinase
Rv0435c	-	ATPase of AAA-family	**Rv2448c**	valS	valyl-tRNA synthase
Rv0436c	pssA	CDP-diacylglycerol-serine o-phosphatidyltransferase	Rv2509	-	putative oxidoreductase
Rv0440	groEL2	60 kD chaperonin 2	Rv2524c	fas	fatty acid synthase
Rv0489	gpm	phosphoglycerate mutase I	Rv2555c	alaS	alanyl-tRNA synthase
Rv0490	senX3	sensor histidine kinase	Rv2580c	hisS	histidyl-tRNA synthase
Rv0500	proC	pyrroline-5-carboxylate reductase	Rv2614c	thrS	threonyl-tRNA synthase
Rv0667	rpoB	[beta] subunit of RNA polymerase	Rv2697c	dut	deoxyuridine triphosphatase
Rv0668	rpoC	[beta]' subunit of RNA polymerase	**Rv2782c**	pepR	protease/peptidase, M16 family (insulinase)
Rv0764c	-	possible lanosterol 14-demethylase cytochrome P450	Rv2793c	truB	tRNA pseudouridine 55 synthase
Rv0861c	-	probable DNA helicase	**Rv2922c**	smc	member of Smc1/Cut3/Cut14 family
Rv1010	ksgA	16S rRNA dimethyltransferase	Rv2925c	rnc	RNAse III
Rv1106c	-	probable cholesterol dehydrogenase	Rv3014c	ligA	DNA ligase
Rv1229c	mrp	similar to MRP/NBP35 ATP-binding proteins	**Rv3025c**	-	NifS-like protein
Rv1239c	corA	probable magnesium and cobalt transport protein	**Rv3080c**	pknK	serine-threonine protein kinase
Rv1294	thrA	homoserine dehydrogenase	Rv3106	fprA	adrenodoxin and NADPH ferredoxin reductase
Rv1323	fadA4	acetyl-CoA C-acetyltransferase (aka thiL)	Rv3255c	manA	mannose-6-phosphate isomerase
Rv1389	gmk	putative guanylate kinase	Rv3264c	rmlA2	glucose-1-phosphate thymidyltransferase
Rv1407	fmu	similar to Fmu protein	**Rv3418c**	groES	10 kD chaperone
Rv1409	ribG	riboflavin biosynthesis	**Rv3490**	otsA	probable [alpha],-trehalose-phosphate synthase
Rv1617	pykA	pyruvate kinase	**Rv3598c**	lysS	lysyl-tRNA synthase
Rv1630	rpsA	30S ribosomal protein S1	**Rv3608c**	folP	dihydropteroate synthase
Rv1745c	-	similar to Q46822 ORF_O182	**Rv3609c**	folE	GTP cyclohydrolase I
Rv1844c	gnd	6-phosphogluconate dehydrogenase (Gram -)	Rv3721c	dnaZX	DNA polymerase III,[gamma] (dnaZ) and t (dnaX)
Rv1981c	nrdF	ribonucleotide reductase small subunit	Rv3834c	serS	seryl-tRNA synthase
Rv2092c	helY	probable helicase, Ski2 subfamily	Rv3907c	pcnA	polynucleotide polymerase

† We follow the Sanger Centre naming convention for MTB genes.

‡ Genes for which high-confidence functional links were found shown in **boldface**

Eight of these were linked to 12 unique MTB genes that satisfied the criteria of the invention's methods (Table 1). Exemplary findings include:

(1) the gene folP, which encodes the enzyme dihydropteroate synthase (DHPS) known to be the target of sulfonamide antibacterial drugs. Although it is found in some eukaryotes, DHPS activity is not found in human cells (see, e.g., Huovinen (1995) Antimicrob. Agents Chemother. 39:279-2890.

(2) the product of the gene folK, a 7,8-dihydro-6-hydroxymethyl-pterinpyrophosphokinase, has recently been proposed as a target for broad-spectrum antibacterial drugs (see, e.g., Stammers (1999) FEBS Lett. 456:49-53).

(3) the gene gpsI, is not only strongly linked to the essential yeast gene pepR, but it is also functionally linked to inhA, the target of the drug isoniazid (see above), making it a very compelling candidate for drug design.

Table 2. Subset of genes from Table 1 that are functionally linked to genes without yeast homologs.

Gene	Link†		Comments
Rv0005	**Rv0002**	dnaN	DNA polymerase III, β-subunit
	Rv0003	recF	DNA replication and SOS induction
	Rv0006	gyrA	DNA gyrase subunit A
Rv0350	**Rv0351**	grpE	stimulates DnaK ATPase activity
	Rv0352	dnaJ	acts with GrpE to stimulate DnaK ATPase
Rv1010	**Rv1008**		Similar to *E.coli* hypothetical protein YcfH
	Rv1009		Possible lipoprotein, similar to various other MTB proteins
	Rv1011		Similar to *E.coli* hypothetical protein YcbH
Rv2439c	Rv2427c	proA	γ-glutamyl phosphate reductase
	Rv2440c	obg	Obg GTP-binding protein
	Rv2441c	rpmA	50S ribosomal protein L27
	Rv2442c	rplU	50S ribosomal protein L21
Rv2782c	**Rv2783c**	gpsI	pppGpp synthase and polyribonucleotide phosphorylase
Rv3598c	**Rv3600c**		similar to *Bacillus subtilis* hypothetical protein YacB
	Rv3606c	folK	7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase
	Rv3607c	folX	may be involved in folate biosynthesis
	Rv3608c‡	folP	dihydropteroate synthase (DHPS)
	Rv3610c	ftsH	inner membrane protein, chaperone
Rv3608c	Rv3598c	lysS	lysyl-tRNA synthase
	Rv3600c		similar to *Bacillus subtilis* hypothetical protein YacB
	Rv3606c	folK	7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase
	Rv3607c	folX	may be involved in folate biosynthesis
	Rv3609c	folE	GTP cyclohydrolase I
	Rv3610c	ftsH	inner membrane protein, chaperone
Rv3609c	**Rv3606c**	folK	7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase
	Rv3607c	folX	may be involved in folate biosynthesis
	Rv3608c‡	folP	dihydropteroate synthase (DHPS)

† Genes without yeast homologs shown in boldface

‡ DHPS activity is found in some eukaryotic cells but not in human cells

In summary, the methods of the invention allowed identification of this combination of functional linkages to essential genes. This information, together with the lack of eukaryotic homologs for these genes, makes this group of proteins promising drug targets, particularly because their inhibition is expected to disrupt vital bacterial processes with a low likelihood of toxicity from the inhibition of a host equivalent.

Computer Implementation

The various techniques, methods, and aspects of the invention described herein can be implemented in part or in whole using computer-based systems and methods. Additionally, computer-based systems and methods can be used to augment or enhance the functionalities and algorithms described herein, increase the speed at which the functions can be performed, and provide additional features and aspects as a part of or in addition to those of the invention described elsewhere in this document. Various exemplary computer-based systems, methods and implementations in accordance with the above-described technology are presented herein.

The processor-based system can include a main memory, such as a random access memory (RAM), and can also include a secondary memory. The secondary memory can include, for example, a hard disk drive and/or a removable storage drive, representing a floppy disk drive, a magnetic tape drive, an optical disk drive, *etc.* The removable storage drive reads from and/or writes to a removable storage medium. Removable storage media can be a floppy disk magnetic tape, an optical disk, and the like, which can be read by and written to by removable storage drive. The removable storage media can includes a computer usable storage medium having stored therein computer software and/or data.

In alternative embodiments, secondary memory may include other similar means for allowing computer programs or other instructions to be loaded into a computer system. Such means can include, for example, a removable storage unit and an interface. Examples of such can include a program cartridge and cartridge interface (such as the found in video game devices), a movable memory chip (such as an EPROM, or PROM) and associated socket, and other removable storage units and interfaces that allow software and data to be transferred from the removable storage unit to the computer system.

The computer system can also include a communications interface. Communications interfaces allow software and data to be transferred between computer system and external devices. Examples of communications interfaces include modems, network interfaces (such as, for example, an Ethernet card), communications ports, PCMCIA slots and cards, and the like. Software and data transferred via a communications interface can be in the form of signals that can be electronic, electromagnetic, optical or other signals capable of being received by a communications interface. These signals can be provided to

communications interface via a channel capable of carrying signals and can be implemented using a wireless medium, wire or cable, fiber optics or other communications medium. Some examples of a channel can include a phone line, a cellular phone link, an RF link, a network interface, and other communications channels.

As used herein, the terms "computer program medium" and "computer usable medium" are used to generally refer to media such as a removable storage device, a disk capable of installation in a disk drive, and signals on a channel, or equivalents thereof. These computer program products are means for providing software or program instructions to computer systems. Computer programs (also called computer control logic) can be stored in main memory and/or secondary memory. Computer programs can also be received via a communications interface. Such computer programs, when executed, enable the computer system to perform the features of the present invention as discussed herein. Computer programs, when executed, enable the processor to perform the features of the present invention. Accordingly, in one aspect of the invention, such computer programs represent controllers of the computer system.

In another aspect of the invention the methods and algorithms are implemented using software, the software may be stored in, or transmitted via, a computer program product and loaded into a computer system using a removable storage drive, hard drive or communications interface. The control logic (software), when executed by the processor, causes the processor to perform the functions of the invention as described herein.

In another aspect, the elements are implemented primarily in hardware using, for example, hardware components such as PALs, application specific integrated circuits (ASICs) or other hardware components. Implementation of a hardware state machine so as to perform the functions described herein will be apparent to person skilled in the relevant art(s). In yet another embodiment, elements are implanted using a combination of both hardware and software.

In another aspect, the computer-based methods can be accessed or implemented over the World Wide Web by providing access via a Web Page to the methods of the present invention. Accordingly, the Web Page is identified by a Universal Resource Locator (URL). The URL denotes both the server machine, and the particular file or page on that machine. In this embodiment, it is envisioned that a consumer or client computer system

interacts with a browser to select a particular URL, which in turn causes the browser to send a request for that URL or page to the server identified in the URL. Typically the server responds to the request by retrieving the requested page, and transmitting the data for that page back to the requesting client computer system (the client/server interaction is typically performed in accordance with the hypertext transport protocol ("HTTP")). The selected page is then displayed to the user on the client's display screen. The client may then cause the server containing a computer program of the present invention to launch an application comprising a method of the invention, for example, to identify a nucleic acid or a polypeptide sequence that may be a target for a drug comprising the steps of (a) providing a first nucleic acid or a polypeptide sequence that is known to be a drug target; (b) providing an algorithm capable analyzing a functional relationship between nucleic acid or polypeptide sequences selected from the group consisting of a "domain fusion" method, a "phylogenetic profile" method and a "physiologic linkage" method; and, (c) comparing the first nucleic acid or the polypeptide drug target sequence to a plurality of sequences using at least one algorithm to identify a second sequence that has a functional relationship to the first sequence, thereby identifying a nucleic acid or a polypeptide sequence that may be a target for a drug, based on a query sequence provided by the client.

Nucleic Acids and Polypeptides

The invention also provides isolated nucleic acids and polypeptides comprising the sequences as set forth in Table 3 and Table 4 (below). As used herein, "isolated," when referring to a molecule or composition, such as, *e.g.*, an isolated infected cell comprising a nucleic acid sequence derived from a library of the invention, means that the molecule or composition (including, e.g., a cell) is separated from at least one other compound, such as a protein, DNA, RNA, or other contaminants with which it is associated *in vivo* or in its naturally occurring state. Thus, a nucleic acid or polypeptide or peptide sequence is considered isolated when it has been isolated from any other component with which it is naturally associated. An isolated composition can, however, also be substantially pure. An isolated composition can be in a homogeneous state. It can be in a dry or an aqueous solution. Purity and homogeneity can be determined, *e.g.*, using any analytical chemistry technique, as described herein.

The term "nucleic acid" or "nucleic acid sequence" refers to a deoxy-ribonucleotide or ribonucleotide oligonucleotide, including single- or double-stranded, or coding or non-coding (e.g., "antisense") forms. The term encompasses nucleic acids, i.e., oligonucleotides, containing known analogues of natural nucleotides. The term also encompasses nucleic-acid-like structures with synthetic backbones, see e.g., Oligonucleotides and Analogues, a Practical Approach, ed. F. Eckstein, Oxford Univ. Press (1991); Antisense Strategies, Annals of the N.Y. Academy of Sciences, Vol 600, Eds. Baserga et al. (NYAS 1992); Milligan (1993) J. Med. Chem. 36:1923-1937; Antisense Research and Applications (1993, CRC Press), WO 97/03211; WO 96/39154; Mata (1997) Toxicol. Appl. Pharmacol. 144:189-197; Strauss-Soukup (1997) Biochemistry 36:8692-8698; Samstag (1996) Antisense Nucleic Acid Drug Dev 6:153-156. As used herein, the "sequence" of a nucleic acid or gene refers to the order of nucleotides in the polynucleotide, including either or both strands (sense and antisense) of a double-stranded DNA molecule, *e.g.*, the sequence of both the coding strand and its complement, or of a single-stranded nucleic acid molecule (sense or antisense). For example, in alternative embodiments, promoters drive the transcription of sense and/or antisense polynucleotide sequences of the invention, as exemplified by Table 3.

The terms "polypeptide," "protein," and "peptide" include compositions of the invention that also include "analogs," or "conservative variants" and "mimetics" ("peptidomimetics") with structures and activity that substantially correspond to the exemplary sequences, such as the sequences in Table 4. Thus, the terms "conservative variant" or "analog" or "mimetic" also refer to a polypeptide or peptide which has a modified amino acid sequence, such that the change(s) do not substantially alter the polypeptide's (the conservative variant's) structure and/or activity (e.g., immunogenicity, ability to bind to human antibodies, etc.), as defined herein. These include conservatively modified variations of an amino acid sequence, i.e., amino acid substitutions, additions or deletions of those residues that are not critical for protein activity, or substitution of amino acids with residues having similar properties (e.g., acidic, basic, positively or negatively charged, polar or non-polar, etc.) such that the substitutions of even critical amino acids does not substantially alter structure and/or activity. Conservative substitution tables providing functionally similar amino acids are well known in the art. For example, one exemplary guideline to select conservative substitutions includes (original residue followed by exemplary substitution):

ala/gly or ser; arg/ lys; asn/ gln or his; asp/glu; cys/ser; gln/asn; gly/asp; gly/ala or pro; his/asn or gln; ile/leu or val; leu/ile or val; lys/arg or gln or glu; met/leu or tyr or ile; phe/met or leu or tyr; ser/thr; thr/ser; trp/tyr; tyr/trp or phe; val/ile or leu. An alternative exemplary guideline uses the following six groups, each containing amino acids that are conservative substitutions for one another: 1) Alanine (A), Serine (S), Threonine (T); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); (see also, e.g., Creighton (1984) Proteins, W.H. Freeman and Company; Schulz and Schimer (1979) Principles of Protein Structure, Springer-Verlag). One of skill in the art will appreciate that the above-identified substitutions are not the only possible conservative substitutions. For example, for some purposes, one may regard all charged amino acids as conservative substitutions for each other whether they are positive or negative. In addition, individual substitutions, deletions or additions that alter, add or delete a single amino acid or a small percentage of amino acids in an encoded sequence can also be considered "conservatively modified variations."

The terms "mimetic" and "peptidomimetic" refer to a synthetic chemical compound that has substantially the same structural and/or functional characteristics of the polypeptides of the invention (e.g., ability to bind, or "capture," human antibodies in an ELISA). The mimetic can be either entirely composed of synthetic, non-natural analogues of amino acids, or, is a chimeric molecule of partly natural peptide amino acids and partly non-natural analogs of amino acids. The mimetic can also incorporate any amount of natural amino acid conservative substitutions as long as such substitutions also do not substantially alter the mimetics' structure and/or activity. As with polypeptides of the invention which are conservative variants, routine experimentation will determine whether a mimetic is within the scope of the invention, i.e., that its structure and/or function is not substantially altered. Polypeptide mimetic compositions can contain any combination of non-natural structural components, which are typically from three structural groups: a) residue linkage groups other than the natural amide bond ("peptide bond") linkages; b) non-natural residues in place of naturally occurring amino acid residues; or c) residues which induce secondary structural mimicry, i.e., to induce or stabilize a secondary structure, e.g., a beta turn, gamma turn, beta sheet, alpha helix conformation, and the like. A polypeptide can be characterized as a

mimetic when all or some of its residues are joined by chemical means other than natural peptide bonds. Individual peptidomimetic residues can be joined by peptide bonds, other chemical bonds or coupling means, such as, e.g., glutaraldehyde, N-hydroxysuccinimide esters, bifunctional maleimides, N,N'-dicyclohexylcarbodiimide (DCC) or N,N'-diisopropylcarbodiimide (DIC). Linking groups that can be an alternative to the traditional amide bond ("peptide bond") linkages include, e.g., ketomethylene (e.g., $-C(=O)-CH_2-$ for $-C(=O)-NH-$), aminomethylene (CH_2-NH), ethylene, olefin (CH=CH), ether (CH_2-O), thioether (CH_2-S), tetrazole (CN_4-), thiazole, retroamide, thioamide, or ester (see, e.g., Spatola (1983) in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp 267-357, "Peptide Backbone Modifications," Marcell Dekker, NY). A polypeptide can also be characterized as a mimetic by containing all or some non-natural residues in place of naturally occurring amino acid residues; non-natural residues are well described in the scientific and patent literature.

The invention comprises nucleic acids comprising sequences as set forth in Table 3, or comprising nucleic acids encoding the polypeptides as set forth in Table 4, operably linked to a transcriptional regulatory sequence. As used herein, the term "operably linked," refers to a functional relationship between two or more nucleic acid (*e.g.*, DNA) segments. Typically, it refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter (defined below) is operably linked to a coding sequence, such as a nucleic acid of the invention, if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, *i.e.*, they are *cis*-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance. For example, in one embodiment, a promoter is operably linked to an ORF-containing nucleic acid sequence of the invention, as exemplified by, e.g., a nucleic acid sequence as set forth in Table 3.

As used herein, the term "promoter" includes all sequences capable of driving transcription of a coding sequence in an expression system. Thus, promoters used in the constructs of the invention include *cis*-acting transcriptional control elements and regulatory

sequences that are involved in regulating or modulating the timing and/or rate of transcription of a nucleic acid of the invention. For example, a promoter can be a *cis*-acting transcriptional control element, including an enhancer, a promoter, a transcription terminator, an origin of replication, a chromosomal integration sequence, 5' and 3' untranslated regions, or an intronic sequence, which are involved in transcriptional regulation. These *cis*-acting sequences typically interact with proteins or other biomolecules to carry out (turn on/off, regulate, modulate, etc.) transcription.

The invention comprises expression cassettes comprising nucleic acids comprising sequences as set forth in Table 3, or comprising nucleic acids encoding the polypeptides as set forth in Table 4. The term "expression vector" refers to any recombinant expression system for the purpose of expressing a nucleic acid sequence of the invention *in vitro* or *in vivo*, constitutively or inducibly, in any cell, including prokaryotic, yeast, fungal, plant, insect or mammalian cell. The term includes linear or circular expression systems. The term includes expression systems that remain episomal or integrate into the host cell genome. The expression systems can have the ability to self-replicate or not, *i.e.*, drive only transient expression in a cell. The term includes recombinant "expression cassettes" which contain only the minimum elements needed for transcription of the recombinant nucleic acid.

Alignment Analysis of Sequences

The nucleic acid and polypeptide sequences of the invention include genes and gene products identified and characterized by sequence identify analysis (i.e., by homology) using the exemplary nucleic acid and protein sequences of the invention, including, e.g., those set forth in Tables 3 and 4. In alternative aspects of the invention, nucleic acids and polypeptides within the scope of the invention include those having 98%, 95%, 90%, 85% or 80% sequence identity (homology) to the exemplary sequences as set forth in Tables 3 and 4.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters are used unless alternative parameters are designated herein. The sequence comparison algorithm then calculates the percent sequence identity for the test

sequence(s) relative to the reference sequence, based on the designated or default program parameters. A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 25 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Natl. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (CLUSTAL, GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection.

In one aspect of the invention (in the methods of the invention, and, to determine if a sequence is within the scope of the invention), a CLUSTAL algorithm is used, e.g., the CLUSTAL W program, see, *e.g.*, Thompson (1994) Nuc. Acids Res. 22:4673-4680; Higgins (1996) Methods Enzymol 266:383-402. Variations can also be used, such as CLUSTAL X, see Jeanmougin (1998) Trends Biochem Sci 23:403-405; Thompson (1997) Nucleic Acids Res 25:4876-4882. In one aspect, the CLUSTAL W program described by Thompson (1994) supra, is used with the following parameters: K tuple (word) size: 1, window size: 5, scoring method: percentage, number of top diagonals: 5, gap penalty: 3, to determine whether a nucleic acid has sufficient sequence identity to an exemplary sequence to be with the scope of the invention. In another aspect, the algorithm PILEUP is used in the methods and to determine whether a nucleic acid has sufficient sequence identity to be with the scope of the invention. This program creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, J. Mol. Evol. 35:351-360 (1987). The method used is similar to the method described by Higgins & Sharp, CABIOS 5:151-153 (1989). Using PILEUP, a

reference sequence (*e.g.*, an exemplary GCA-associated sequence of the invention) is compared to another sequence to determine the percent sequence identity relationship (*i.e.*, that the second sequence is substantially identical and within the scope of the invention) using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. In one embodiment, PILEUP obtained from the GCG sequence analysis software package, *e.g.*, version 7.0 (Devereaux(1984) *Nuc. Acids Res.* 12:387-395), using the parameters described therein, is used in the methods and to identify nucleic acids within the scope of the invention. In a another aspect, a BLAST algorithm is used (in the methods, e.g., to determine percent sequence identity (*i.e.*, substantial similarity or identity) and whether a nucleic acid is within the scope of the invention), see, e.g., Altschul (1990) *J. Mol. Biol.* 215:403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information, NIH. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul (1990) *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues, always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. In one embodiment, to determine if a nucleic acid sequence is within the scope of the invention, the BLASTN program (for nucleotide sequences) is used incorporating as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as default parameters a wordlength (W) of 3, an expectation (E) of 10, and the

BLOSUM62 scoring matrix (see, *e.g.*, Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

Hybridization for Identifying Nucleic Acids of the Invention

Nucleic acids within the scope of the invention include isolated or recombinant nucleic acids that specifically hybridize under stringent hybridization conditions to an exemplary nucleic acid of the invention (including a sequence encoding an exemplary polypeptide) as set forth in Tables 3 and 4. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in, e.g., Tijssen (1993) infra. Generally, stringent conditions are selected to be about 5 to 10°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

For selective or specific hybridization, a positive signal (*e.g.*, identification of a nucleic acid of the invention) is about 10 times background hybridization. "Stringent" hybridization conditions that are used to identify substantially identical nucleic acids within the scope of the invention include hybridization in a buffer comprising 50% formamide, 5x SSC, and 1% SDS at 42°C, or hybridization in a buffer comprising 5x SSC and 1% SDS at 65°C, both with a wash of 0.2x SSC and 0.1% SDS at 65°C. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, and 1% SDS at 37°C, and a wash in 1X SSC at 45°C. Those of ordinary skill will readily recognize that alternative but comparable hybridization and wash conditions can be utilized to provide conditions of similar stringency. Nucleic acids which do not hybridize to each other under stringent hybridization conditions are still substantially identical if the

polypeptides which they encode are substantially identical. This may occur, *e.g.*, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code, as discussed herein (see discussion on "conservative substitutions"). However, the selection of a hybridization format is not critical - it is the stringency of the wash conditions that set forth the conditions that determine whether a nucleic acid is within the scope of the invention. Wash conditions used to identify nucleic acids within the scope of the invention include, e.g.: a salt concentration of about 0.02 molar at pH 7 and a temperature of at least about 50°C or about 55°C to about 60°C; or, a salt concentration of about 0.15 M NaCl at 72°C for about 15 minutes; or, a salt concentration of about 0.2X SSC at a temperature of at least about 50°C or about 55°C to about 60°C for about 15 to about 20 minutes; or, the hybridization complex is washed twice with a solution with a salt concentration of about 2X SSC containing 0.1% SDS at room temperature for 15 minutes and then washed twice by 0.1X SSC containing 0.1% SDS at 68°C for 15 minutes; or, equivalent conditions. See Sambrook, Tijssen and Ausubel (see below) for a description of SSC buffer and equivalent conditions.

General Techniques

The nucleic acid and polypeptide sequences of the invention and other nucleic acids used to practice this invention, whether RNA, cDNA, genomic DNA, vectors, viruses or hybrids thereof, may be isolated from a variety of sources, genetically engineered, amplified, and/or expressed recombinantly. Any recombinant expression system can be used, including, in addition to bacterial cells, e.g., mammalian, yeast, insect or plant cell expression systems.

Alternatively, these nucleic acids and polypeptides can be synthesized *in vitro* by well-known chemical synthesis techniques, as described in, e.g., Carruthers (1982) Cold Spring Harbor Symp. Quant. Biol. 47:411-418; Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896; Narang (1979) Meth. Enzymol. 68:90; Brown (1979) Meth. Enzymol. 68:109; Beaucage (1981) Tetra. Lett. 22:1859; U.S. Patent No. 4,458,066.

Techniques for the manipulation of nucleic acids, such as, e.g., generating mutations in sequences, subcloning, labeling probes, sequencing, hybridization and the like

are well described in the scientific and patent literature, see, e.g., Sambrook, ed., MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

Polypeptides and peptides of the invention can also be synthesized, whole or in part, using chemical methods well known in the art. See e.g., Caruthers (1980) Nucleic Acids Res. Symp. Ser. 215-223; Horn (1980) Nucleic Acids Res. Symp. Ser. 225-232; Banga, A.K., Therapeutic Peptides and Proteins, Formulation, Processing and Delivery Systems (1995) Technomic Publishing Co., Lancaster, PA. For example, peptide synthesis can be performed using various solid-phase techniques (see e.g., Roberge (1995) Science 269:202; Merrifield (1997) Methods Enzymol. 289:3-13) and automated synthesis may be achieved, e.g., using the ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

The skilled artisan will recognize that individual synthetic residues and polypeptides incorporating mimetics can be synthesized using a variety of procedures and methodologies, which are well described in the scientific and patent literature, *e.g.*, Organic Syntheses Collective Volumes, Gilman, et al. (Eds) John Wiley & Sons, Inc., NY. Polypeptides incorporating mimetics can also be made using solid phase synthetic procedures, as described, *e.g.*, by Di Marchi, et al., U.S. Pat. No. 5,422,426. Peptides and peptide mimetics of the invention can also be synthesized using combinatorial methodologies. Various techniques for generation of peptide and peptidomimetic libraries are well known, and include, *e.g.*, multipin, tea bag, and split-couple-mix techniques; see, *e.g.*, al-Obeidi (1998) Mol. Biotechnol. 9:205-223; Hruby (1997) Curr. Opin. Chem. Biol. 1:114-119; Ostergaard (1997) Mol. Divers. 3:17-27; Ostresh (1996) Methods Enzymol. 267:220-234. Modified peptides of the invention can be further produced by chemical modification methods, see, *e.g.*, Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896.

Peptides and polypeptides of the invention can also be synthesized and expressed as fusion proteins with one or more additional domains linked thereto for, *e.g.*,

producing a more immunogenic peptide, to more readily isolate a recombinantly synthesized peptide, to identify and isolate antibodies and antibody-expressing B cells, and the like. Detection and purification facilitating domains include, *e.g.*, metal chelating peptides such as polyhistidine tracts and histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor Xa or enterokinase (Invitrogen, San Diego CA) between the purification domain and GCA-associated peptide or polypeptide can be useful to facilitate purification. For example, an expression vector can include an epitope-encoding nucleic acid sequence linked to six histidine residues followed by a thioredoxin and an enterokinase cleavage site (see *e.g.*, Williams (1995) Biochemistry 34:1787-1797; Dobeli (1998) Protein Expr. Purif. 12:404-414). The histidine residues facilitate detection and purification while the enterokinase cleavage site provides a means for purifying the epitope from the remainder of the fusion protein. Technology pertaining to vectors encoding fusion proteins and application of fusion proteins are well described in the scientific and patent literature, see *e.g.*, Kroll (1993) DNA Cell. Biol., 12:441-53.

The invention provides antibodies that specifically bind to the polypeptides of the invention, as set forth in Table 4. These antibodies can be useful in the screening methods of the invention. The polypeptides or peptide can be conjugated to another molecule or can be administered with an adjuvant. The coding sequence can be part of an expression cassette or vector capable of expressing the immunogen *in vivo*. (see, *e.g.*, Katsumi (1994) Hum. Gene Ther. 5:1335-9). Methods of producing polyclonal and monoclonal antibodies are known to those of skill in the art and described in the scientific and patent literature, see, *e.g.*, Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY, Wiley/Greene, NY (1991); Stites (eds.) BASIC AND CLINICAL IMMUNOLOGY (7th ed.) Lange Medical Publications, Los Altos, CA; Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY (1986); Harlow (1988) ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Publications, New York.

Antibodies also can be generated *in vitro*, *e.g.*, using recombinant antibody binding site expressing phage display libraries, in addition to the traditional *in vivo* methods using animals. See, *e.g.*, Huse (1989) Science 246:1275; Ward (1989) Nature 341:544;

Hoogenboom (1997) Trends Biotechnol. 15:62-70; Katz (1997) Annu. Rev. Biophys. Biomol. Struct. 26:27-45. Human antibodies can be generated in mice engineered to produce only human antibodies, as described by, e.g., U.S. Patent No. 5,877,397; 5,874,299; 5,789,650; and 5,939,598. B-cells from these mice can be immortalized using standard techniques (e.g., by fusing with an immortalizing cell line such as a myeloma or by manipulating such B-cells by other techniques to perpetuate a cell line) to produce a monoclonal human antibody-producing cell. See, e.g., U.S. Patent No. 5,916,771; 5,985,615.

TABLE 3

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>emb|AL123456|MTBH37RV:1127087-1127881, Rv1008 SEQ ID NO:37
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>Rv1009 - lipoprotein, similar to various other MTB proteins TB.seq 1128089:1129174 MW:38079
>emb|AL123456|MTBH37RV:1128089-1129177, Rv1009 SEQ ID NO:38
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>Rv1010 ksgA 16S rRNA dimethyltransferase TB.seq 1129150:1130100 MW:34647
>emb|AL123456|MTBH37RV:1129150-1130103, ksgA SEQ ID NO:39
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>Rv1011- Homology to E.coli protein YcbH TB.seq 1130189:1131106 MW:31350
>emb|AL123456|MTBH37RV:1130189-1131109, Rv1011 SEQ ID NO:40
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>Rv1106c - cholesterol dehydrogenase TB.seq 1232845:1233954 MW:40743
>emb|AL123456|MTBH37RV:c1233954-1232842, Rv1106c SEQ ID NO:41
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>Rv1110 lytB2 TB.seq 1236183:1237187 MW:36298
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>Rv1216c - TB.seq 1359473:1360144 MW:24863
>emb|AL123456|MTBH37RV:c1360144-1359470, Rv1216c SEQ ID NO:43
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>Rv1223 htrA TB.seq 1365810:1367456 MW:56547
>emb|AL123456|MTBH37RV:1365810-1367459, htrA SEQ ID NO:44
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>Rv1224 - TB.seq 1367461:1367853 MW:14083
>emb|AL123456|MTBH37RV:1367461-1367856, Rv1224 SEQ ID NO:45
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>Rv1229c mrp similar to MRP/NBP35 ATP-binding proteins TB.seq 1371778:1372947 MW:41064
>emb|AL123456|MTBH37RV:c1372947-1371775, mrp SEQ ID NO:46
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>emb|AL123456|MTBH37RV:c1383040-1381940, corA SEQ ID NO:47
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>emb|AL123456|MTBH37RV:1485860-1487029, fadA4 SEQ ID NO:50
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>Rv1389 gmk putative guanylate kinase TB.seq 1564399:1565022 MW:22064
>emb|AL123456|MTBH37RV:1564399-1565025, gmk SEQ ID NO:51
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>emb|AL123456|MTBH37RV:1583099-1584472, fmu SEQ ID NO:52
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>emb|AL123456|MTBH37RV:1585192-1586211, ribG SEQ ID NO:53
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>emb|AL123456|MTBH37RV:1617715-1618068, secG SEQ ID NO:54
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>Rv1617 pykA pyruvate kinase TB.seq 1816187:1817602 MW:50668
>emb|AL123456|MTBH37RV:1816187-1817605, pykA SEQ ID NO:56
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>emb|AL123456|MTBH37RV:1833540-1834985, rpsA SEQ ID NO:57
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>Rv1745c - similar to Q46822 ORF_O182 TB.seq 1971381:1971989 MW:22490
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>emb|AL123456|MTBH37RV:2039451-2041418, PPE SEQ ID NO:61
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>Rv3834c serS seryl-tRNA synthase TB.seq 4307655:4308911 MW:45293
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TABLE 4

>Rv0002 dnaN DNA polymerase III, b-subunit TB.seq 2052:3257 MW:42114 SEQ ID NO:147
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VPAKTLAEAAKAGIGGSDVRLSLGTGPGVGKDGLLGISGNGKRSTTRLLDAEFPKFRQLLPTEHTAVA
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>Rv0003 recF DNA replication and SOS induction TB.seq 3280:4434 MW:42181 SEQ ID NO:148
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>Rv0005 gyrB DNA gyrase subunit B TB.seq 5123:7264 MW:78441 SEQ ID NO:149
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>Rv0018c ppp TB.seq 21640:23181 MW:53781 SEQ ID NO:154
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>Rv0019c - TB.seq 23273:23737 MW:17153 SEQ ID NO:155

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>Rv0020c - TB.seq 23864:25444 MW:56881 SEQ ID NO:156

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SEQ ID NO:157

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>Rv0051 - TB.seq 55694:57373 MW:61210 SEQ ID NO:159
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>Rv0106 - TB.seq 124372:125565 MW:43701 SEQ ID NO:160
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>Rv0125 - TB.seq 151146:152210 MW:34927 SEQ ID NO:161
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SVTWQTKSGGTRTGNVTLAEGPPA

>Rv0350 dnaK 70 kD heat shock protein, chromosome replication TB.seq 419833:421707 MW:66832 SEQ ID NO:162

MARAVGIDLGTTNSVVSVLEGGDPVVVANSEGSRTTPSIVAFARNGEVLVGQPAKNQAVTNVDRTV RSVKRHMGSDWSIEIDGKKYTAPEISARILMKLKRDAEAYLGEDITDAVITTPAYFNDAQRQATKDAG QIAGLNVLRIVNEPTAAALAYGLDKGEKEQRILVFDLGGGTFDVSLLEIGEGVVEVRATSGDNHLGGD DWDQRVVDWLVDKFKGTSGIDLTKDKMAMQRLREAAEKAKIELSSSQSTSINLPYITVDADKNPLFLD EQLTRAEFQRITQDLLDRTRKPFQSVIADTGISVSEIDHVVLVGGSTRMPAVTDLVKELTGGKEPNKG VNPDEVVAVGAALQAGVLKGEVKDVLLLDVTPLSLGIETKGGVMTRLIERNTTIPTKRSETFTTADDN QPSVQIQVYQGEREIAAHNKLLGSFELTGIPPAPRGIPQIEVTFDIDANGIVHVTAKDKGTGKENTIRIQ EGSGLSKEDIDRMIKDAEAHAEEDRKRREEADVRNQAETLVYQTEKFVKEQREAEGGSKVPEDTLN KVDAAVAEAKAALGGSDISAIKSAMEKLGQESQALGQAIYEAAQAASQATGAAHPGGEPGGAHPGS ADDVVDAEVVDDGREAK

>Rv0351 grpE stimulates DnaK ATPase activity TB.seq 421707:422411 MW:24501
SEQ ID NO:163
VTDGNQKPDGNSGEQVTVTDKRRIDPETGEVRHVPPGDMPGGTAAADAAHTEDKVAELTADLQRV QADFANYRKRALRDQQAAADRAKASVVSQLLGVLDDLERARKHGDLESGPLKSVADKLDSALTGLG LVAFGAEGEDFDPVLHEAVQHEGDGGQGSKPVIGTVMRQGYQLGEQVLRHALVGVVDTVVVDAAE LESVDDGTAVADTAENDQADQGNSADTSGEQAESEPSGS

>Rv0352 dnaJ acts with GrpE to stimulate DnaK ATPase TB.seq 422450:423634 MW:41346
SEQ ID NO:164
MAQREWVEKDFYQELGVSSDASPEEIKRAYRKLARDLHPDANPGNPAAGERFKAVSEAHNVLSDPA KRKEYDETRRLFAGGGFGGRRFDSGFGGGFGGFGVGGDGAEFNLNDLFDAASRTGGTTIGDLFGG LFGRGGSARPSRPRRGNDLETETELDFVEAAKGVAMPLRLTSPAPCTNCHGSGARPGTSPKVCPTC NGSGVINRNQGAFGFSEPCTDCRGSGSIIEHPCEECKGTGVTTRTRTINVRIPPGVEDGQRIRLAGQ GEAGLRGAPSGDLYVTVHVRPDKIFGRDGDDLTVTVPVSFTELALGSTLSVPTLDGTVGVRVPKGTA DGRILRVRGRGVPKRSGGSGDLLVTVKVAVPPNLAGAAQEALEAYAAAERSSGFNPRAGWAGNR
>Rv0363c fba fructose bisphosphate aldolase TB.seq 441266:442297 MW:36545
SEQ ID NO:165
MPIATPEVYAEMLGQAKQNSYAFPAINCTSSETVNAAIKGFADAGSDGIIQFSTGGAEFGSGLGVKDM VTGAVALAEFTHVIAAKYPVNVALHTDHCPKDKLDSYVRPLLAISAQRVSKGGNPLFQSHMWDGSAV PIDENLAIAQELLKAAAAAKIILEIEIGVVGGEEDGVANEINEKLYTSPEDFEKTIEALGAGEHGKYLLAA TFGNVHGVYKPGNVKLRPDILAQGQQVAAAKLGLPADAKPFDFVFHGGSGSLKSEIEEALRYGVVKM NVDTDTQYAFTRPIAGHMFTNYDGVLKVDGEVGVKKVYDPRSYLKKAEASMSQRVVQACNDLHCA GKSLTH
>Rv0405 pks6 TB.seq 485729:489934 MW:147615 SEQ ID NO:166
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KCAITAYPDRGFTNAGTFAESGGFLKDVAGFDNRFFDIPPDEALRMDPQQRLLLEVSWEALEHAGIIP
ESLRLSRTGVFVGVSSTDYVRLVSASAQQKSTIWDNTGGSSSIIANRISYFLDIQGPSIVIDTACSSSLV
AVHLACRSLSTWDCDIALVGGTNVLISPEPWGGFREAGILSQTGCCHAFDKSADGMVRGEGCGVIVL
QRLSDARLEGRRILAILTGSAVNQDGKSNGIMAPNPSAQIGVLENACKSARVDPLEIGYVEAHGTGTS
LGDRIEAHALGMVFGRKRPGSGPLMIGSIKPNIGHLEGAAGIAGLIKAVLMVERGSLLPSGGFTEPNP
AIPFTELGLRVVDELQEWPVVAGRPRRAGVSSFGFGGTNAHVIVEEAGSVGADTVSGRADVGGSGG
GVVAWVISGKTASALAAQAGRLGRYVRARPALDVVDVGYSLVSTRSVFDHRAVVVGQTRDELLAGL
AGVVAGRPEAGVVCGVGKPAGKTAFVFAGQGSQWLGMGSELYAAYPVFAEALDAVVDELDRHLRY
PLRDVIWGHDQDLLNTTEFAQPALFAVEVALYRLLMSWGVRPGLVLGHSVGELAAAHVAGALCLPD
AAMLVAARGRLMQALPAGGAMFAVQAREDEVAPMLGHDVSIAAVNGPASVVISGAHDAVSAIADRL
RGQGRRVHRLAVSHAFHSALMEPMIAEFTAVAAELSVGLPTIPVISNVTGQLVADDFASADYWARHIR
AVVRFGDSVRSAHCAGASRFIEVGPGGGLTSLIEASLADAQIVSVPTLRKDRPEPVSVMTAAAQGFV
SGMGLDWASVFSGYRPKRVELPTYAFQHQKFWLAPAPSVSDPTAAGQIGASDGGAELLASSGFAA
RLAGRSADEQLAAAIEVVCEHAAAVLGRDGAAGLDAGQAFADSGFNSLSAVELRNRLTAVTAVTLPA
TAIFDHPTPTELAQYLITQIDGHGSSAAAAANPAERIDALTDLFLQACDAGRDADGWKMVALASNTRE
RMSSPVRNNVSKNVALLADGISDVVVICIPTLTVLSDQREYRDIANAMTGRHSVYSLTLPGFDSSDAL
PQNADMIVETVSNAIIDVVGGSCRFVLSGYSSGGVLAYALCSHLSVKHQRNPLGVALIDTYLPSQIAN
PSMNEGFSPNDTGKGLSREVIRVARMLNRLTATRLTAAATYAAIFQAWEPGRSMAPVLNIVAKDRIAT
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>Rv0435c - ATPase of AAA-family TB.seq 522348:524531 MW:75315 SEQ ID NO:167
VTHPDPARQLTLTARLNTSAVDSRRGVVRLHPNAIAALGIREWDAVSLTGSRTTAAVAGLAAADTAV
GTVLLDDVTLSNAGLREGTEVIVSPVTVYGARSVTLSGSTLATQSVPPVTLRQALLGKVMTVGDAVSL
LPRDLGPGTSTSAASRALAAAVGISWTSELLTVTGVDPDGPVSVQPNSLVTWGAGVPAAMGTSTAG
QVSISSPEIQIEELKGAQPQAAKLTEWLKLALDEPHLLQTLGAGTNLGVLVSGPAGVGKATLVRAVCD
GRRLVTLDGPEIGALAAGDRVKAVASAVQAVRHEGGVLLITDADALLPAAAEPVASLILSELRTAVATA
GVVLIATSARPDQLDARLRSPELCDRELGLPLPDAATRKSLLEALLNPVPTGDLNLDEIASRTPGFVVA
DLAALVREAALRAASRASADGRPPMLHQDDLLGALTVIRPLSRSASDEVTVGDVTLDDVGDMAAAK
QALTEAVLWPLQHPDTFARLGVEPPRGVLLYGPPGCGKTFVVRALASTGQLSVHAVKGSELMDKWV
GSSEKAVRELFRRARDSAPSLVFLDELDALAPRRGQSFDSGVSDRVVAALLTELDGIDPLRDVVMLG
ATNRPDLIDPALLRPGRLERLVFVEPPDAAARREILRTAGKSIPLSSDVDLDEVAAGLDGYSAADCVAL
LREAALTAMRRSIDAANVTAADLATARETVRASLDPLQVASLRKFGTKGDLRS

>Rv0436c pssA CDP-diacylglycerol-serine o-phosphatidyltransferase TB.seq 524531:525388 MW:31219 SEQ ID NO:168
MIGKPRGRRGVNLQILPSAMTVLSICAGLTAIKFALEHQPKAAMALIAAAAILDGLDGRVARILDAQSR
MGAEIDSLADAVNFGVTPALVLYVSMLSKWPVGWVVVLLYAVCVVLRLARYNALQDDGTQPAYAHE

FFVGMPAPAGAVSMIGLLALKMQFGEGWWTSGWFLSFWVTGTSILLVSGIPMKKMHAVSVPPNYAA
ALLAVLAICAAAAVLAPYLLIWVIIIAYMCHIPFAVRSQRWLAQHPEVWDDKPKQRRAVRRASRRAHP
YRPSMARLGLRKPGRRL

>Rv0440 groEL 260 kD chaperonin 2 TB.seq 528606:530225 MW:56728 SEQ ID NO:169
MAKTIAYDEEARRGLERGLNALADAVKVTLGPKGRNVVLEKKWGAPTITNDGVSIAKEIELEDPYEKI
GAELVKEVAKKTDDVAGDGTTTATVLAQALVREGLRNVAAGANPLGLKRGIEKAVEKVTETLLKGAK
EVETKEQIAATAAISAGDQSIGDLIAEAMDKVGNEGVITVEESNTFGLQLELTEGMRFDKGYISGYFVT
DPERQEAVLEDPYILLVSSKVSTVKDLLPLLEKVIGAGKPLLIIAEDVEGEALSTLVVNKIRGTFKSVAVK
APGFGDRRKAMLQDMAILTGGQVISEEVGLTLENADLSLLGKARKVVVTKDETTIVEGAGDTDAIAGR
VAQIRQEIENSDSDYDREKLQERLAKLAGGVAVIKAGAATEVELKERKHRIEDAVRNAKAAVEEGIVA
GGGVTLLQAAPTLDELKLEGDEATGANIVKVALEAPLKQIAFNSGLEPGVVAEKVRNLPAGHGLNAQT
GVYEDLLAAGVADPVKVTRSALQNAASIAGLFLTTEAVVADKPEKEKASVPGGGDMGGMDF

>Rv0482 murB TB.seq 570537:571643 MW:38522 SEQ ID NO:170
MKRSGVGSLFAGAHIAEAVPLAPLTTLRVGPIARRVITCTSAEQVVAALRHLDSAAKTGADRPLVFAG
GSNLVIAENLTDLTVVRLANSGITIDGNLVRAEAGAVFDDVVVRAIEQGLGGLECLSGIPGSAGATPVQ
NVGAYGAEVSDTITRVRLLDRCTGEVRWVSARDLRFGYRTSVLKHADGLAVPTVVLEVEFALDPSGR
SAPLRYGELIAALNATSGERADPQAVREAVLALRARKGMVLDPTDHDTWSVGSFFTNPVVTQDVYE
RLAGDAATRKDGPVPHYPAPDGVKLAAGWLVERAGFGKGYPDAGAAPCRLSTKHALALTNRGGAT
AEDVVTLARAVRDGVHDVFGITLKPEPVLIGCML

>Rv0483 - TB.seq 571708:573060 MW:47859 SEQ ID NO:171
VVIRVLFRPVSLIPVNNSSTPQSQGPISRRLALTALGFGVLAPNVLVACAGKVTKLAEKRPPPAPRLTF
RPADSAADVVPIAPISVEVGDGWFQRVALTNSAGKVVAGAYSRDRTIYTITEPLGYDTTYTWSGSAV
GHDGKAVPVAGKFTTVAPVKTINAGFQLADGQTVGIAAPVIIQFDSPISDKAAVERALTVTTDPPVEGG
WAWLPDEAQGARVHWRPREYYPAGTTVDVDAKLYGLPFGDGAYGAQDMSLHFQIGRRQVVKAEV
SSHRIQVVTDAGVIMDFPCSYGEADLARNVTRNGIHVVTEKYSDFYMSNPAAGYSHIHERWAVRISN
NGEFIHANPMSAGAQGNSNVTNGCINLSTENAEQYYRSAVYGDPVEVTGSSIQLSYADGDIWDWAV
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>Rv0489 gpm phosphoglycerate mutase I TB.seq 578424:579170 MW:27217 SEQ ID NO:172
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AHLALDSADRLWIPVRRSWRLNERHYGALQGLDKAETKARYGEEQFMAWRRSYDTPPPPIERGSQ
FSQDADPRYADIGGGPLTECLADVVARFLPYFTDVIVGDLRVGKTVLIVAHGNSLRALVKHLDQMSDD
EIVGLNIPTGIPLRYDLDSAMRPLVRGGTYLDPEAAAAGAAAVAGQGRG

>Rv0490 senX 3sensor histidine kinase TB.seq 579347:580576 MW:44794 SEQ ID NO:173
VTVFSALLLAGVLSALALAVGGAVGMRLTSRVVEQRQRVATEWSGITVSQMLQCIVTLMPLGAAVVD
THRDVVYLNERAKELGLVRDRQLDDQAWRAARQALGGEDVEFDLSPRKRSATGRSGLSVHGHARL
LSEEDRRFAVVFVHDQSDYARMEAARRDFVANVSHELKTPVGAMALLAEALLASADDSETVRRFAE
KVLIEANRLGDMVAELIELSRLQGAERLPNMTDVDVDTIVSEAISRHKVAADNADIEVRTDAPSNLRVL
GDQTLLVTALANLVSNAIAYSPRGSLVSISRRRRGANIEIAVTDRGIGIAPEDQERVFERFFRGDKARS
RATGGSGLGLAIVKHVAANHDGTIRVWSKPGTGSTFTLALPALIEAYHDDERPEQAREPELRSNRSQ
REEELSR

>Rv0500 proC pyrroline-5-carboxylate reductase TB.seq 590081:590965 MW:30172
SEQ ID NO:174
MLFGMARIAIIGGGSIGEALLSGLLRAGRQVKDLVVAERMPDRANYLAQTYSVLVTSAADAVENATFV
VVAVKPADVEPVIADLANATAAAENDSAEQVFVTVVAGITIAYFESKLPAGTPVVRAMPNAAALVGAG
VTALAKGRFVTPQQLEEVSALFDAVGGVLTVPESQLDAVTAVSGSGPAYFFLLVEALVDAGVGVGLS
RQVATDLAAQTMAGSAAMLLERMEQDQGGANGELMGLRVDLTASRLRAAVTSPGGTTAAALRELE
RGGFRMAVDAAVQAAKSRSEQLRITPE

>Rv0528 - TB.seq 618303:619889 MW:57132 SEQ ID NO:175
MWRSLTSMGTALVLLFLLALAAIPGALLPQRGLNAAKVDDYLAAHPLIGPWLDELQAFDVFSSFWFTA
IYVLLFVSLVGCLAPRTIEHARSLRATPVAAPRNLARLPKHAHARLAGEPAALAATITGRLRGWRSITR
QQGDSVEVSAEKGYLREFGNLVFHFALLGLLVAVAVGKLFGYEGNVIVIADGGPGFCSASPAAFDSF
RAGNTVDGTSLHPICVRVNNFQAHYLPSGQATSFAADIDYQADPATADLIANSWRPYRLQVNHPLRV
GGDRVYLQGHGYAPTFTVTFPDGQTRTSTVQWRPDNPQTLLSAGVVRIDPPAGSYPNPDERRKHQI
AIQGLLAPTEQLDGTLLSSRFPALNAPAVAIDIYRGDTGLDSGRPQSLFTLDHRLIEQGRLVKEKRVNL
RAGQQVRIDQGPAAGTVVRFDGAVPFVNLQVSHDPGQSWVLVFAITMMAGLLVSLLVRRRRVWARI
TPTTAGTVNVELGGLTRTDNSGWGAEFERLTGRLLAGFEARSPDMAEAAAGTGRDVD

>Rv0667 rpoB [beta] subunit of RNA polymerase TB.seq 759805:763320 MW:129220
SEQ ID NO:176
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GAWLEFDVDKRDTVGVRIDRKRRQPVTVLLKALGWTSEQIVERFGFSEIMRSTLEKDNTVGTDEALL
DIYRKLRPGEPPTKESAQTLLENLFFKEKRYDLARVGRYKVNKKLGLHVGEPITSSTLTEEDVVATIEY
LVRLHEGQTTMTVPGGVEVPVETDDIDHFGNRRLRTVGELIQNQIRVGMSRMERVVRERMTTQDVE
AITPQTLINIRPVVAAIKEFFGTSQLSQFMDQNNPLSGLTHKRRLSALGPGGLSRERAGLEVRDVHPS
HYGRMCPIETPEGPNIGLIGSLSVYARVNPFGFIETPYRKVVDGVVSDEIVYLTADEEDRHVVAQANS

PIDADGRFVEPRVLVRRKAGEVEYVPSSEVDYMDVSPRQMVSVATAMIPFLEHDDANRALMGANMQ
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HGTCANQCPIVDAGDRVEAGQVIADGPCTDDGEMALGKNLLVAIMPWEGHNYEDAIILSNRLVEEDV
LTSIHIEEHEIDARDTKLGAEEITRDIPNISDEVLADLDERGIVRIGAEVRDGDILVGKVTPKGETELTPE
ERLLRAIFGEKAREVRDTSLKVPHGESGKVIGIRVFSREDEDELPAGVNELVRVYVAQKRKISDGDKL
AGRHGNKGVIGKILPVEDMPFLADGTPVDIILNTHGVPRRMNIGQILETHLGWCAHSGWKVDAAKGV
PDWAARLPDELLEAQPNAIVSTPVFDGAQEAELQGLLSCTLPNRDGDVLVDADGKAMLFDGRSGEP
FPYPVTVGYMYIMKLHHLVDDKIHARSTGPYSMITQQPLGGKAQFGGQRFGEMECWAMQAYGAAY
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DLERAAANLGINLSRNESASVEDLA

>Rv0668 rpoC [beta]' subunit of RNA polymerase TB.seq 763368:767315 MW:146740
SEQ ID NO:177
VLDVNFFDELRIGLATAEDIRQWSYGEVKKPETINYRTLKPEKDGLFCEKIFGPTRDWECYCGKYKRV
RFKGIICERCGVEVTRAKVRRERMGHIELAAPVTHIWYFKGVPSRLGYLLDLAPKDLEKIIYFAAYVITS
VDEEMRHNELSTLEAEMAVERKAVEDQRDGELEARAQKLEADLAELEAEGAKADARRKVRDGGER
EMRQIRDRAQRELDRLEDIWSTFTKLAPKQLIVDENLYRELVDRYGEYFTGAMGAESIQKLIENFDIDA
EAESLRDVIRNGKGQKKLRALKRLKVVAAFQQSGNSPMGMVLDAVPVIPPELRPMVQLDGGRFATS
DLNDLYRRVINRNNRLKRLIDLGAPEIIVNNEKRMLQESVDALFDNGRRGRPVTGPGNRPLKSLSDLL
KGKQGRFRQNLLGKRVDYSGRSVIVVGPQLKLHQCGLPKLMALELFKPFVMKRLVDLNHAQNIKSAK
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MAVHLPLSAEAQAEARILMLSSNNILSPASGRPLAMPRLDMVTGLYYLTTEVPGDTGEYQPASGDHP
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NELLPLGYPFVNKQMHKKVQAAIINDLAERYPMIVVAQTVDKLKDAGFYWATRSGVTVSMADVLVPP
RKKEILDHYEERADKVEKQFQRGALNHDERNEALVEIWKEATDEVGQALREHYPDDNPIITIVDSGAT
GNFTQTRTLAGMKGLVTNPKGEFIPRPVKSSFREGLTVLEYFINTHGARKGLADTALRTADSGYLTRR
LVDVSQDVIVREHDCQTERGIVVELAERAPDGTLIRDPYIETSAYARTLGTDAVDEAGNVIVERGQDL
GDPEIDALLAAGITQVKVRSVLTCATSTGVCATCYGRSMATGKLVDIGEAVGIVAAQSIGEPGTQLTM
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GVSIHDKHIEVIVRQMLRRVTIIDSGSTEFLPGSLIDRAEFEAENRRVVAEGGEPAAGRPVLMGITKAS
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>Rv0711 atsA TB.seq 806333:808693 MW:86216 SEQ ID NO:178
MAPEATEAFNGTIELDIRDSEPDWGPYAAPVAPEHSPNILYLVWDDVGIATWDCFGGLVEMPAMTRV
AERGVRLSQFHTTALCSPTRASLLTGRNATTVGMATIEEFTDGFPNCNGRIPADTALLPEVLAEHGYN

TYCVGKWHLTPLEESNMASTKRHWPTSRGFERFYGFLGGETDQWYPDLVYDNHPVSPPGTPEGG
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RQKALGIVPPDTELSPINPYLDVPGPNGETWPLQDTVRPWDSLSDEEKKLFCRMAEVFAGFLSYTDA
QIGRILDYLEESGQLDNTIIVVISDNGASGEGGPNGSVNEGKFFNGYIDTVAESMKLFDHLGGPQTYN
HYPIGWAMAFNTPYKLFKRYASHEGGIADPAIISWPNGIAAHGEIRDNYVNVSDITPTVYDLLGMTPP
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RWELFHIAADRSQCHDLAAEHPDKLEELKALWFSEAAKYNGLPLADLNLLETMTRSRPYLVSERASY
VYYPDCADVGIGAAVEIRGRSFAVLADVTIDTTGAEGVLFKHGGAHGGHVLFVRDGRLHYVYNFLGE
RQQLVSSSGPVPSGRHLLGVRYLRTGTVPNSHTPVGDLELFFDENLVGALTNVLTHPGTFGLAGAAI
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>Rv0764c - lanosterol 14-demethylase cytochrome P450 TB.seq 856683:858035 MW:50879
SEQ ID NO:179
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GDDDLDQAKAYPFMTPIFGEGVVFDASPERRKEMLHNAALRGEQMKGHAATIEDQVRRMIADWGE
AGEIDLLDFFAELTIYTSSACLIGKKFRDQLDGRFAKLYHELERGTDPLAYVDPYLPIESFRRRDEARN
GLVALVADIMNGRIANPPTDKSDRDMLDVLIAVKAETGTPRFSADEITGMFISMMFAGHHTSSGTASW
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HRIHEGDLVAASPAISNRIPEDFPDPHDFVPARYEQPRQEDLLNRWTWIPFGAGRHRCVGAAFAIMQI
KAIFSVLLREYEFEMAQPPESYRNDHSKMVVQLAQPACVRYRRRTGV

>Rv0861c - DNA helicase TB.seq 958524:960149 MW:59773 SEQ ID NO:180
VQSDKTVLLEVDHELAGAARAAIAPFAELERAPEHVHTYRITPLALWNARAAGHDAEQVVDALVSYS
RYAVPQPLLVDIVDTMARYGRLQLVKNPAHGLTLVSLDRAVLEEVLRNKKIAPMLGARIDDDTVVVHP
SERGRVKQLLLKIGWPAEDLAGYVDGEAHPISLHQEGWQLRDYQRLAADSFWAGGSGVVVLPCGA
GKTLVGAAAMAKAGATTLILVTNIVAARQWKRELVARTSLTENEIGEFSGERKEIRPVTISTYQMITRR
TKGEYRHLELFDSRDWGLIIYDEVHLLPAPVFRMTADLQSKRRLGLTATLIREDGREGDVFSLIGPKR
YDAPWKDIEAQGWIAPAECVEVRVTMTDSERMMYATAEPEERYRICSTVHTKIAVVKSILAKHPDEQ
TLVIGAYLDQLDELGAELGAPVIQGSTRTSEREALFDAFRRGEVATLVVSKVANFSIDLPEAAVAVQVS
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AI

>Rv0904c accD3 TB.seq 1006694:1008178 MW:51741 SEQ ID NO:181
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VACEFDFLGGSIGVAAAERITAAVERATAERLPLLASPSSGGTRMQEGTVAFLQMVKIAAAIQLHNQA
RLPYLVYLRHPTTGGVFASWGSLGHLTVAEPGALIGFLGPRVYELLYGDPFPSGVQTAENLRRHGIID
GVVALDRLRPMLDRALTVLIDAPEPLPAPQTPAPVPDVPTWDSVVASRRPDRPGVRQLLRHGATDR

VLLSGTDQGEAATTLLALARFGGQPTVVLGQQRAVGGGGSTVGPAALREARRGMALAAELCLPLVL
VIDAAGPALSAAAEQGGLAGQIAHCLAELVTLDTPTVSILLGQGSGGPALAMLPADRVLAALHGWLAP
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>Rv0983 - TB.seq 1099064:1100455 MW:46454 SEQ ID NO:182
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GPSGGPVAASAAPSIPAANMPPGSVEQVAAKVVPSVVMLETDLGRQSEEGSGIILSAEGLILTNNHVI
AAAAKPPLGSPPPKTTVTFSDGRTAPFTVVGADPTSDIAVVRVQGVSGLTPISLGSSSDLRVGQPVLA
IGSPLGLEGTVTTGIVSALNRPVSTTGEAGNQNTVLDAIQTDAAINPGNSGGALVNMNAQLVGVNSAI
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>Rv1008 - Similar to E.coli protein YcfH TB.seq 1127087:1127878 MW:29066 SEQ ID NO:183
LVDAHTHLDACGARDADTVRSLVERAAAAGVTAVVTVADDLESARWVTRAAEWDRRVYAAVALHPT
RADALTDAARAELERLVAHPRVVAVGETGIDMYWPGRLDGCAEPHVQREAFAWHIDLAKRTGKPLM
IHNRQADRDVLDVLRAEGAPDTVILHCFSSDAAMARTCVDAGWLLSLSGTVSFRTARELREAVPLMP
VEQLLVETDAPYLTPHPHRGLANEPYCLPYTVRALAELVNRRPEEVALITTSNARRAYGLGWMRQ

>Rv1009 - lipoprotein, similar to various other MTB proteins TB.seq 1128089:1129174 MW:38079 SEQ ID NO:184
MLRLVVGALLLVLAFAGGYAVAACKTVTLTVDGTAMRVTTMKSRVIDIVEENGFSVDDRDDLYPAAG
VQVHDADTIVLRRSRPLQISLDGHDAKQVWTTASTVDEALAQLAMTDTAPAAASRASRVPLSGMALP
VVSAKTVQLNDGGLVRTVHLPAPNVAGLLSAAGVPLLQSDHVVPAATAPIVEGMQIQVTRNRIKKVTE
RLPLPPNARRVEDPEMNMSREVVEDPGVPGTQDVTFAVAEVNGVETGRLPVANVVVTPAHEAVVR
VGTKPGTEVPPVIDGSIWDAIAGCEAGGNWAINTGNGYYGGVQFDQGTWEANGGLRYAPRADLAT
REEQIAVAEVTRLRQGWGAWPVCAARAGAR

>Rv1010 ksgA 16S rRNA dimethyltransferase TB.seq 1129150:1130100 MW:34647 SEQ ID NO:185
MCCTSGCALTIRLLGRTEIRRLAKELDFRPRKSLGQNFVHDANTVRRVVAASGVSRSDLVLEVGPGL
GSLTLALLDRGATVTAVEIDPLLASRLQQTVAEHSHSEVHRLTVVNRDVLALRREDLAAAPTAVVANL
PYNVAVPALLHLLVEFPSIRVVTVMVQAEVAERLAAEPGSKEYGVPSVKLRFFGRVRRCGMVSPTVF
WPIPRVYSGLVRIDRYETSPWPTDDAFRRRVFELVDIAFAQRRKTSRNAFVQWAGSGSESANRLLAA
SIDPARRGETLSIDDFVRLLRRSGGSDEATSTGRDARAPDISGHASAS

>Rv1011 - Similar to E.coli protein YcbH TB.seq 1130189:1131106 MW:31350
SEQ ID NO:186
VPTGSVTVRVPGKVNLYLAVGDRREDGYHELTTVFHAVSLVDEVTVRNADVLSLELVGEGADQLPTD
ERNLAWQAAELMAEHVGRAPDVSIMIDKSIPVAGGMAGGSADAAAVLVAMNSLWELNVPRRDLRML
AARLGSDVPFALHGGTALGTGRGEELATVLSRNTFHWVLAFADSGLLTSAVYNELDRLREVGDPPRL
GEPGPVLAALAAGDPDQLAPLLGNEMQAAAVSLDPALARALRAGVEAGALAGIVSGSGPTCAFLCTS
ASSAIDVGAQLSGAGVCRTVRVATGPVPGARVVSAPTEV

>Rv1106c - cholesterol dehydrogenase TB.seq 1232845:1233954 MW:40743 SEQ ID NO:187
MLRRMGDASLTTELGRVLVTGGAGFVGANLVTTLLDRGHWVRSFDRAPSLLPAHPQLEVLQGDITD
ADVCAAAVDGIDTIFHTAAIIELMGGASVTDEYRQRSFAVNVGGTENLLHAGQRAGVQRFVYTSSNS
VVMGGQNIAGGDETLPYTDRFNDLYTETKVVAERFVLAQNGVDGMLTCAIRPSGIWGNGDQTMFRK
LFESVLKGHVKVLVGRKSARLDNSYVHNLIHGFILAAAHLVPDGTAPGQAYFINDAEPINMFEFARPVL
EACGQRWPKMRISGPAVRWVMTGWQRLHFRFGFPAPLLEPLAVERLYLDNYFSIAKARRDLGYEPL
FTTQQALTECLPYYVSLFEQMKNEARAEKTAATVKP

>Rv1110 lytB2 TB.seq 1236183:1237187 MW:36298 SEQ ID NO:188
MVPTVDMGIPGASVSSRSVADRPNRKRVLLAEPRGYCAGVDRAVETVERALQKHGPPVYVRHEIVH
NRHVVDTLAKAGAVFVEETEQVPEGAIVVFSAHGVAPTVHVSASERNLQVIDATCPLVTKVHNEARR
FARDDYDILLIGHEGHEEVVGTAGEAPDHVQLVDGVDAVDQVTVRDEDKVVWLSQTTLSVDETMEIV
GRLRRRFPKLQDPPSDDICYATQNRQVAVKAMAPECELVIVVGSRNSSNSVRLVEVALGAGARAAH
LVDWADDIDSAWLDGVTTVGVTSGASVPEVLVRGVLERLAECGYDIVQPVTTANETLVFALPRELRS
PR

>Rv1216c - TB.seq 1359473:1360144 MW:24863 SEQ ID NO:189
MHIGLKIFIWGVLGLVVFGALLFGPAGTFDYWQAWVFLAAFVSTTIGPTIYLARNDPAALQRRMRSGP
LAEGRTIQKFIVIGAFLGFFAMMVLSACDHRYGWSSVPAAVCVIGDVLVMTGLGIAMLVVIQNRYAAS
TVRVEAGQILASDGLYKIVRHPMYAGNVVMMTGIPLALGSYWAMFILVPGTLVLVFRILDEEKLLTQEL
SGYREYRQLVRYRLVPYVW

>Rv1223 htrA TB.seq 1365810:1367456 MW:56547 SEQ ID NO:190
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RPVFRPPVDPASRQAFGRPSGVQGSFVAERVRPQKYQDQSDFTPNDQLADPVLQEAFGRPFAGAE
SLQRHPIDAGALAAEKDGAGPDEPDDPWRDPAAAAALGTPALAAPAPHGALAGSGKLGVRDVLFGG
KVSYLALGILVAIALVIGGIGGVIGRKTAEVVDAFTTSKVTLSTTGNAQEPAGRFTKVAAAVADSVVTIE
SVSDQEGMQGSGVIVDGRGYIVTNNHVISEAANNPSQFKTTVVFNDGKEVPANLVGRDPKTDLAVLK
VDNVDNLTVARLGDSSKVRVGDEVLAVGAPLGLRSTVTQGIVSALHRPVPLSGEGSDTDTVIDAIQTD

ASINHGNSGGPLIDMDAQVIGINTAGKSLSDSASGLGFAIPVNEMKLVANSLIKDGKIVHPTLGISTRSV
SNAIASGAQVANVKAGSPAQKGGILENDVIVKVGNRAVADSDEFVVAVRQLAIGQDAPIEVVREGRH
VTLTVKPDPDST

>Rv1224 - TB.seq 1367461:1367853 MW:14083 SEQ ID NO:191
VFANIGWWEMLVLVMVGLVVLGPERLPGAIRWAASALRQARDYLSGVTSQLREDIGPEFDDLRGHL
GELQKLRGMTPRAALTKHLLDGDDSLFTGDFDRPTPKKPDAAGSAGPDATEQIGAGPIPFDSDAT

>Rv1229c mrp similar to MRP/NBP35 ATP-binding proteins TB.seq 1371778:1372947 MW:41064
SEQ ID NO:192
MPSRLHSAVMSGTRDGDLNAAIRTALGKVIDPELRRPITELGMVKSIDTGPDGSVHVEIYLTIAGCPKK
SEITERVTRAVADVPGTSAVRVSLDVMSDEQRTELRKQLRGDTREPVIPFAQPDSLTRVYAVASGKG
GVGKSTVTVNLAAAMAVRGLSIGVLDADIHGHSIPRMMGTTDRPTQVESMILPPIAHQVKVISIAQFTQ
GNTPVVWRGPMLHRALQQFLADVYWGDLDVLLLDLPPGTGDVAISVAQLIPNAELLVVTTPQLAAAE
VAERAGSIALQTRQRIVGVVENMSGLTLPDGTTMQVFGEGGGRLVAERLSRAVGADVPLLGQIPLDP
ALVAAGDSGVPLVLSSPDSAIGKELHSIADGLSTRRRGLAGMSLGLDPTRR

>Rv1239c corA magnesium and cobalt transport protein TB.seq 1381943:1383040 MW:41470
SEQ ID NO:193
VFPGFDALPEVLRPVARPQPPNAHPVAQPPAQALVDCGVYVCGQRLPGKYTYAAALREVREIELTG
QEAFVWIGLHEPDENQMQDVADVFGLHPLAVEDAVHAHQRPKLERYDETLFLVLKTVNYVPHESVV
LAREIVKTGEIMIFVGKDFVVTVRHGEHGGLSEVRKRMDADPEHLRLGPYAVMHAIADYVVDHYLEVT
NLMETDIDSIEEVAFAPGRKLDIEPIYLLKREVVELRRCVNPLSTAFQRMQTESKDLISKEVRRYLRDV
ADHQTEAADQIASYDDMLNSLVQAALARVGMQQNMDMRKISAWAGIIAVPTMIAGIYGMNFHFMPEL
DSRWGYPTVIGGMVLICLFLYHVFRNRNWL
>Rv1279 - TB.seq 1430060:1431643 MW:57332 SEQ ID NO:194
MDTQSDYVVVGTGSAGAVVASRLSTDPATTVVALEAGPRDKNRFIGVPAAFSKLFRSEIDWDYLTEP
QPELDGREIYWPRGKVLGGSSSMNAMMWVRGFASDYDEWAARAGPRWSYADVLGYFRRIENVTA
AWHFVSGDDSGVTGPLHISRQRSPRSVTAAWLAAARECGFAAARPNSPRPEGFCETVVTQRRGAR
FSTADAYLKPAMRRKNLRVLTGATATRVVIDGDRAVGVEYQSDGQTRIVYARREVVLCAGAVNSPQL
LMLSGIGDRDHLAEHDIDTVYHAPEVGCNLLDHLVTVLGFDVEKDSLFAAEKPGQLISYLLRRRGMLT
SNVGEAYGFVRSRPELKLPDLELIFAPAPFYDEALVPPAGHGVVFGPILVAPQSRGQITLRSADPHAK
PVIEPRYLSDLGGVDRAAMMAGLRICARIAQARPLRDLLGSIARPRNSTELDEATLELALATCSHTLYH
PMGTCRMGSDEASVVDPQLRVRGVDGLRVADASVMPSTVRGHTHAPSVLIGEKAADLIRS
>Rv1294 thrA homoserine dehydrogenase TB.seq 1449373:1450695 MW:45522 SEQ ID NO:195
VPGDEKPVGVAVLGLGNVGSEVVRIIENSAEDLAARVGAPLVLRGIGVRRVTTDRGVPIELLTDDIEEL
VAREDVDIVVEVMGPVEPSRKAILGALERGKSVVTANKALLATSTGELAQAAESAHVDLYFEAAVAGA

IPVIRPLTQSLAGDTVLRVAGIVNGTTNYILSAMDSTGADYASALADASALGYAEADPTADVEGYDAA AKAAILASIAFHTRVTADDVYREGITKVTPADFGSAHALGCTIKLLSICERITTDEGSQRVSARVYPALV PLSHPLAAVNGAFNAVVVEAEAAGRLMFYGQGAGGAPTASAVTGDLVMAARNRVLGSRGPRESKY AQLPVAPMGFIETRYYVSMNVADKPGVLSAVAAEFAKREVSIAEVRQEGVVDEGGRRVGARIVVVTH LATDAALSETVDALDDLDVVQGVSSVIRLEGTGL

>Rv1323 fadA4 acetyl-CoA C-acetyltransferase (aka thiL) TB.seq 1485860:1487026 MW:40049 SEQ ID NO:196

VIVAGARTPIGKLMGSLKDFSASELGAIAIKGALEKANVPASLVEYVIMGQVLTAGAGQMPARQAAVA AGIGWDVPALTINKMCLSGIDAIALADQLIRAREFDVVVAGGQESMTKAPHLLMNSRSGYKYGDVTVL DHMAYDGLHDVFTDQPMGALTEQRNDVDMFTRSEQDEYAAASHQKAAAAWKDGVFADEVIPVNIP QRTGDPLQFTEDEGIRANTTAAALAGLKPAFRGDGTITAGSASQISDGAAAVVVMNQEKAQELGLTW LAEIGAHGVVAGPDSTLQSQPANAINKALDREGISVDQLDVVEINEAFAAVALASIRELGLNPQIVNVN GGAIAVGHPLGMSGTRITLHAALQLARRGSGVGVAALCGAGGQGDALILRAG

>Rv1389 gmk putative guanylate kinase TB.seq 1564399:1565022 MW:22064 SEQ ID NO:197

VSVGEGPDTKPTARGQPAAVGRVVVLSGPSAVGKSTVVRCLRERIPNLHFSVSATTRAPRPGEVDG VDYHFIDPTRFQQLIDQGELLEWAEIHGGLHRSGTLAQPVRAAAATGVPVLIEVDLAGARAIKKTMPE AVTVFLAPPSWQDLQARLIGRGTETADVIQRRLDTARIELAAQGDFDKVVVNRRLESACAELVSLLVG TAPGSP

>Rv1407 fmu similar to Fmu protein TB.seq 1583099:1584469 MW:48494 SEQ ID NO:198

MTPRSRGPRRRPLDPARRAAFETLRAVSARDAYANLVLPALLAQRGIGGRDAAFATELTYGTCRAR GLLDAVIGAAAERSPQAIDPVLLDLLRLGTYQLLRTRVDAHAAVSTTVEQAGIEFDSARAGFVNGVLR TIAGRDERSWVGELAPDAQNDPIGHAAFVHAHPRWIAQAFADALGAAVGELEAVLASDDERPAVHLA ARPGVLTAGELARAVRGTVGRYSPFAVYLPRGDPGRLAPVRDGQALVQDEGSQLVARALTLAPVDG DTGRWLDLCAGPGGKTALLAGLGLQCAARVTAVEPSPHRADLVAQNTRGLPVELLRVDGRHTDLDP GFDRVLVDAPCTGLGALRRRPEARWRRQPADVAALAKLQRELLSAAIALTRPGGVVLYATCSPHLAE TVGAVADALRRHPVHALDTRPLFEPVIAGLGEGPHVQLWPHRHGTDAMFAAALRRLT

>Rv1409 ribG riboflavin biosynthesis TB.seq 1585192:1586208 MW:35367 SEQ ID NO:199

MNVEQVKSIDEAMGLAIEHSYQVKGTTYPKPPVGAVIVDPNGRIVGAGGTEPAGGDHAEVVALRRAG GLAAGAIVVVTMEPCNHYGKTPPCVNALIEARVGTVVYAVADPNGIAGGGAGRLSAAGLQVRSGVLA EQVAAGPLREWLHKQRTGLPHVTWKYATSIDGRSAAADGSSQWISSEAARLDLHRRRAIADAILVGT GTVLADDPALTARLADGSLAPQQPLRVVVGKRDIPPEARVLNDEARTMMIRTHEPMEVLRALSDRTD VLLEGGPTLAGAFLRAGAINRILAYVAPILLGGPVTAVDDVGVSNITNALRWQFDSVEKVGPDLLLSLV AR

>Rv1440 secG TB.seq 1617715:1618065 MW:12140 SEQ ID NO:200
VAGVTAAVSARLKADEARRPGFYAAGSGPLPQVRGSTLPVMELALQITLIVTSVLVVLLVLLHRAKGG
GLSTLFGGGVQSSLSGSTVVEKNLDRLTLFVTGIWLVSIIGVALLIKYR

>Rv1484 inhA TB.seq 1674200:1675006 MW:28529 SEQ ID NO:201
MTGLLDGKRILVSGIITDSSIAFHIARVAQEQGAQLVLTGFDRLRLIQRITDRLPAKAPLLELDVQNEEH
LASLAGRVTEAIGAGNKLDGVVHSIGFMPQTGMGINPFFDAPYADVSKGIHISAYSYASMAKALLPIM
NPGGSIVGMDFDPSRAMPAYNWMTVAKSALESVNRFVAREAGKYGVRSNLVAAGPIRTLAMSAIVG
GALGEEAGAQIQLLEEGWDQRAPIGWNMKDATPVAKTVCALLSDWLPATTGDIIYADGGAHTQLL

>Rv1617 pykA pyruvate kinase TB.seq 1816187:1817602 MW:50668 SEQ ID NO:202
VTRRGKIVCTLGPATQRDDLVRALVEAGMDVARMNFSHGDYDDHKVAYERVRVASDATGRAVGVL
ADLQGPKIRLGRFASGATHWAEGETVRITVGACEGSHDRVSTTYKRLAQDAVAGDRVLVDDGKVAL
VVDAVEGDDVVCTVVEGGPVSDNKGISLPGMNVTAPALSEKDIEDLTFALNLGVDMVALSFVRSPAD
VELVHEVMDRIGRRVPVIAKLEKPEAIDNLEAIVLAFDAVMVARGDLGVELPLEEVPLVQKRAIQMARE
NAKPVIVATQMLDSMIENSRPTRAEASDVANAVLDGADALMLSGETSVGKYPLAAVRTMSRIICAVEE
NSTAAPPLTHIPRTKRGVISYAARDIGERLDAKALVAFTQSGDTVRRLARLHTPLPLLAFTAWPEVRS
QLAMTWGTETFIVPKMQSTDGMIRQVDKSLLELARYKRGDLVVIVAGAPPGTVGSTNLIHVHRIGEDD
V

>Rv1630 rpsA 30S ribosomal protein S1 TB.seq 1833540:1834982 MW:53203 SEQ ID NO:203
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HDVDPNEVVSVGDEVEALVLTKEDKEGRLILSKKRAQYERAWGTIEALKEKDEAVKGTVIEVVKGGLI
LDIGLRGFLPASLVEMRRVRDLQPYIGKEIEAKIIELDKNRNNVVLSRRAWLEQTQSEVRSEFLNNLQK
GTIRKGVVSSIVNFGAFVDLGGVDGLVHVSELSWKHIDHPSEVVQVGDEVTVEVLDVDMDRERVSLS
LKATQEDPWRHFARTHAIGQIVPGKVTKLVPFGAFVRVEEGIEGLVHISELAERHVEVPDQVVAVGDD
AMVKVIDIDLERRRISLSLKQANEDYTEEFDPAKYGMADSYDEQGNYIFPEGFDAETNEWLEGFEKQ
RAEWEARYAEAERRHKMHTAQMEKFAAAEAAGRGADDQSSASSAPSEKTAGGSLASDAQLAALRE
KLAGSA

>Rv1631 - TB.seq 1835011:1836231 MW:44669 SEQ ID NO:204
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LAAKAFRDDESRGVLNGIVHPLVARRRSEIIAAVSGDAVVVEDIPLLVESGMAPLFPLVVVVHADVELR
VRRLVEQRGMAEADARARIAAQASDQQRRAVADVWLDNSGSPEDLVRRARDVWNTRVQPFAHNL
AQRQIARAPARLVPADPSWPDQARRIVNRLKIACGHKALRVDHIGSTAVSGFPDFLAKDVIDIQVTVE
SLDVADELAEPLLAAGYPRLEHITQDTEKTDARSTVGRYDHTDSAALWHKRVHASADPGRPTNVHLR

VHGWPNQQFALLFVDWLAANPGAREDYLTVKCDADRRADGELARYVTAKEPWFLDAYQRAWEWA DAVHWRP

>Rv1706c - TB.seq 1932695:1933876 MW:39779 SEQ ID NO:205

MTLDVPVNQGHVPPGSVACCLVGVTAVADGIAGHSLSNFGALPPEINSGRMYSGPGSGPLMAAAAA WDGLAAELSSAATGYGAAISELTNMRWWSGPASDSMVAAVLPFVGWLSTTATLAEQAAMQARAAA AAFEAAFAMTVPPPAIAANRTLLMTLVDTNWFGQNTPAIATTESQYAEMWAQDAAAMYGYASAAAP ATVLTPFAPPPQTTNATGLVGHATAVAALRGQHSWAAAIPWSDIQKYWMMFLGALATAEGFIYDSG GLTLNALQFVGGMLWSTALAEAGAAEAAAGAGGAAGWSAWSQLGAGPVAASATLAAKIGPMSVPP GWSAPPATPQAQTVARSIPGIRSAAEAAETSVLLRGAPTPGRSRAAHMGRRYGRRLTVMADRPNVG

>Rv1745c - similar to Q46822 ORF_O182 TB.seq 1971381:1971989 MW:22490 SEQ ID NO:206

MTRSYRPAPPIERVVLLNDRGDATGVADKATVHTGDTPLHLAFSSYVFDLHDQLLITRRAATKRTWP AVWTNSCCGHPLPGESLPGAIRRRLAAELGLTPDRVDLILPGFRYRAAMADGTVENEICPVYRVQVD QQPRPNSDEVDAIRWLSWEQFVRDVTAGVIAPVSPWCRSQLGYLTKLGPCPAQWPVADDCRLPKA AHGN

>Rv1800 - TB.seq 2039451:2041415 MW:67068 SEQ ID NO:207

MLPNFAVLPPEVNSARVFAGAGSAPMLAAAAAWDDLASELHCAAMSFGSVTSGLVVGWWQGSASA AMVDAAASYIGWLSTSAAHAEGAAGLARAAVSVFEEALAATVHPAMVAANRAQVASLVASNLFGQN APAIAALESLYECMWAQDAAAMAGYYVGASAVATQLASWLQRLQSIPGAASLDARLPSSAEAPMGV VRAVNSAIAANAAAAQTVGLVMGGSGTPIPSARYVELANALYMSGSVPGVIAQALFTPQGLYPVVVIK NLTFDSSVAQGAVILESAIRQQIAAGNNVTVFGYSQSATISSLVMANLAASADPPSPDELSFTLIGNPN NPNGGVATRFPGISFPSLGVTATGATPHNLYPTKIYTIEYDGVADFPRYPLNFVSTLNAIAGTYYVHSN YFILTPEQIDAAVPLTNTVGPTMTQYYIIRTENLPLLEPLRSVPIVGNPLANLVQPNLKVIVNLGYGDPA YGYSTSPPNVATPFGLFPEVSPVVIADALVAGTQQGIGDFAYDVSHLELPLPADGSTMPSTAPGSGT PVPPLSIDSLIDDLQVANRNLANTISKVAATSYATVLPTADIANAALTIVPSYNIHLFLEGIQQALKGDPM GLVNAVGYPLAADVALFTAAGGLQLLIIISAGRTIANDISAIVP

>Rv1844c gnd 6-phosphogluconate dehydrogenase (Gram -) TB.seq 2093732:2095186 MW:51548 SEQ ID NO:208

MSSSESPAGIAQIGVTGLAVMGSNIARNFARHGYTVAVHNRSVAKTDALLKEHSSDGKFVRSETIPEF LAALEKPRRVLIMVKAGEATDADAVINELADAMEPGDIIIDGGNALYTDTMRREKAMRERGLHFVGAG ISGGEEGALNGPSIMPGGPAESYQSLGPLLEEISAHVDGVPCCTHIGPDGSGHFVKMVHNGIEYSDM QLIGEAYQLMRDGLGLTAPAIADVFTEWNNGDLDSYLVEITAEVLRQTDAKTGKPLVDVIVDRAEQKG TGRWTVKSALDLGVPVTGIAEAVFARALSGSVGQRSAASGLASGKLGEQPADPATFTEDVRQALYA SKIVAYAQGFNQIQAGSAEFGWDITPGDLATIWRGGCIIRAKFLNHIKEAFDASPNLASLIVAPYFRGA

VESAIDSWRRVVSTAAQLGIPTPGFSSALSYYDALRTARLPAALTQAQRDFFGAHTYGRIDEPGKFHT
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>Rv1900c lipJ TB.seq 2146246:2147631 MW:49685 SEQ ID NO:209
VAQAPHIHRTRYAKCGDMDIAYQVLGDGPTDLLVLPGPFVPIDSIDDEPSLYRFHRRLASFSRVIRLDH
RGVGLSSRLAAITTLGPKFWAQDAIAVMDAVGCEQATIFAPSFHAMNGLVLAADYPERVRSLIVVNGS
ARPLWAPDYPVGAQVRRADPFLTVALEPDAVERGFDVLSIVAPTVAGDDVFRAWWDLAGNRAGPP
SIARAVSKVIAEADVRDVLGHIEAPTLILHRVGSTYIPVGHGRYLAEHIAGSRLVELPGTDTLYWVGDT
GPMLDEIEEFITGVRGGADAERMLATIMFTDIVGSTQHAAALGDDRWRDLLDNHDTIVCHEIQRFGGR
EVNTAGDGFVATFTSPSAAIACADDIVDAVAALGIEVRIGIHAGEVEVRDASHGTDVAGVAVHIGARVC
ALAGPSEVLVSSTVRDIVAGSRHRFAERGEQELKGVPGRWRLCVLMRDDATRTR

>Rv1967 - TB.seq 2210599:2211624 MW:36516 SEQ ID NO:210
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RISINPDATVRVQFTADNSVTLTRGTRAVIRYDNLFGDRYLALEEGAGGLAVLRPGHTIPLARTQPALD
LDALIGGFKPLFRALNPEQVNALSEQLLHAFAGQGPTIGSLLAQSAAVTNTLADRDRLIGQVITNLNVV
LGSLGAHTDRLDQAVTSLSALIHRLAQRKTDISNAVAYTNAAAGSVADLLSQARAPLAKVVRETDRVA
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PK

>Rv1975 - TB.seq 2218050:2218712 MW:23650 SEQ ID NO:211
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TLDLLNNRHLNDDTGSDGSTPQDRAHAAGFRGKVAETVAINPAVAISGIELINQWYYNPAFFAIMSDC
ANTQIGVWSENSPDRTVVVAVYGQPDRPSAMPPRGAVTGPPSPVAAQENVPIDPSPDYDASDEIEY
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>Rv1981c nrdF ribonucleotide reductase small subunit TB.seq 2224221:2225186 MW:36591
SEQ ID NO:212
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QRKAQIIVDYYRGDDALKRKASSVMLESFLFYSGFYLPMYWSSRGKLTNTADLIRLIIRDEAVHGYYIG
YKCQRGLADLTDAERADHREYTCELLHTLYANEIDYAHDLYDELGWTDDVLPYMRYNANKALANLG
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>Rv2092c helY helicase, Ski2 subfamily TB.seq 2349335:2352052 MW:99576 SEQ ID NO:213
VTELAELDRFTAELPFSLDDFQQRACSALERGHGVLVCAPTGAGKTVVGEFAVHLALAAGSKCFYTT
PLKALSNQKHTDLTARYGRDQIGLLTGDLSVNGNAPVVVMTTEVLRNMLYADSPALQGLSYVVMDE

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LVGKRMFDLFDYRIGEAEGQPQVNRELLRHIAHRREADRMADWQPRRRGSGRPGFYRPPGRPEVI
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REGLLRGLAAHHAGMLPAFRHTVEELFTAGLVKAVFATETLALGINMPARTVVLERLVKFNGEQHMP
LTPGEYTQLTGRAGRRGIDVEGHAVVIWHPEIEPSEVAGLASTRTFPLRSSFAPSYNMTINLVHRMGP
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SRLQRRQAATDALAALRRGDIITITHGRRGGLAVVLESARDRDDPRPLVLTEHRWAGRISSADYSGTT
PVGSMTLPKRVEHRQPRVRRDLASALRSAAAGLVIPAARRVSEAGGFHDPELESSREQLRRHPVHT
SPGLEDQIRQAERYLRIERDNAQLERKVAAATNSLARTFDRFVGLLTEREFIDGPATDPVVTDDGRLL
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>Rv2101 helZ helicase, Snf2/Rad54 family TB.seq 2360238:2363276 MW:111632
SEQ ID NO:214
MLVLHGFWSNSGGMRLWAEDSDLLVKSPSQALRSARPHPFAAPADLIAGIHPGKPATAVLLLPSLRS
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VERGRVLPQLRRDTHGAAACWRPVLQGRDVVAMTSLVSAMPPVCRAEVGGHDPHELATSALDAMV
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GPARATFRLSEVETENEETPAGSLWRLEFLLQSTQDPSLLVPAEQAWNDDGSLRRWLDRPQELLLT
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YTPVDGVVGKASKFGREQLVEFRWELAVGDDPLSEEEIAALTETKSPLIRLRGQWVALDTEQMRRGL
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AVKNSLSRAAKAVRRLRAAHRVALTGTPMENRLAELWSIMDFLNPGLLGSSERFRTRYAIPIERHGHT
EPAERLRASTRPYILRRLKTDPAIIDDLPEKIEIKQYCQLTTEQASLYQAVVADMMEKIENTEGIERRGN
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>Rv2144c - TB.seq 2404166:2404519 MW:12028 SEQ ID NO:217
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>Rv2146c - TB.seq 2405667:2405954 MW:10805 SEQ ID NO:218
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>Rv2147c - TB.seq 2406119:2406841 MW:27630 SEQ ID NO:219
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>Rv2148c - TB.seq 2406841:2407614 MW:27694 SEQ ID NO:220
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>Rv2150c ftsZ TB.seq 2408386:2409522 MW:38757 SEQ ID NO:221
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>Rv2152c murC TB.seq 2410639:2412120 MW:51146 SEQ ID NO:222
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>Rv2153c murG TB.seq 2412120:2413349 MW:41829 SEQ ID NO:223
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>Rv2154c ftsW TB.seq 2413349:2414920 MW:56306 SEQ ID NO:224
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>Rv2156c murX TB.seq 2416397:2417473 MW:37714 SEQ ID NO:226
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>Rv2157c murF TB.seq 2417473:2419002 MW:51634 SEQ ID NO:227
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>Rv2159c - TB.seq 2420632:2421663 MW:36377 SEQ ID NO:229
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>Rv2197c - TB.seq 2461505:2462146 MW:22481 SEQ ID NO:233
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>Rv2199c - TB.seq 2463234:2463650 MW:14866 SEQ ID NO:235
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>Rv2200c ctaC TB.seq 2463661:2464749 MW:40449 SEQ ID NO:236
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>Rv2439c proB glutamate 5-kinase TB.seq 2737118:2738245 MW:38789 SEQ ID NO:239
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>Rv2442c rplU 50S ribosomal protein L21 TB.seq 2740048:2740359 MW:11152
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>Rv2448c valS valyl-tRNA synthase TB.seq 2747596:2750223 MW:97822 SEQ ID NO:243
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>Rv2482c plsB2 TB.seq 2786915:2789281 MW:88284 SEQ ID NO:244
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>Rv2509 - putative oxidoreductase TB.seq 2824676:2825479 MW:28014 SEQ ID NO:245
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>Rv2524c fas fatty acid synthase TB.seq 2840124:2849330 MW:326226 SEQ ID NO:246
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>Rv3014c ligA DNA ligase TB.seq 3372545:3374617 MW:75258 SEQ ID NO:263

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>Rv3025c - NifS-like protein TB.seq 3383885:3385063 MW:40948 SEQ ID NO:264

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>Rv3080c pknK serine-threonine protein kinase TB.seq 3442656:3445985 MW:119420
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>Rv3106 fprA adrenodoxin and NADPH ferredoxin reductase TB.seq 3474004:3475371
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>Rv3235 - TB.seq 3611296:3611934 MW:22659 SEQ ID NO:267
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>Rv3255c manA mannose-6-phosphate isomerase TB.seq 3635040:3636263 MW:43340
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FGVEVMANSDNVLRGGLTPKHVDVPELLRVLDFAPTPKARLRPPIRREGLGLVFETPTDEFAATLLVL
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ATVGL

>Rv3264c rmlA2 glucose-1-phosphate thymidyltransferase TB.seq 3644897:3645973 MW:37840
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LLADGDCKIYGYVDASYWRDMGTPEDFVRGSADLVRGIAPSPALRGHRGEQLVHDGAAVSPGALLI
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>Rv3368c - TB.seq 3780334:3780975 MW:23734 SEQ ID NO:270
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>Rv3382c lytB1 TB.seq 3796447:3797433 MW:34667 SEQ ID NO:271
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RFPTLGQPPSEDICYATTNRQRALQSMVGECDVVLVIGSCNSSNSRRLVELAQRSGTPAYLIDGPDDI
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>Rv3418c groES 10 kD chaperone TB.seq 3836985:3837284 MW:10773 SEQ ID NO:272
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>Rv3423c alr TB.seq 3840193:3841416 MW:43357 SEQ ID NO:273

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>Rv3490 otsA [alpha],-trehalose-phosphate synthase TB.seq 3908232:3909731 MW:55864 SEQ ID NO:274

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VVQLATPSRERVESYQTLRNDIERQVGHINGEYGEVGHPVVHYLHRPAPRDELIAFFVASDVMLVTP
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>Rv3598c lysS lysyl-tRNA synthase TB.seq 4041423:4042937 MW:55678 SEQ ID NO:275

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DCWRIAAKSLRPLPVAHKEMSEESRVRQRYVDLIVRPEARAVARLRIAVVRAIRTALQRRGFLEVETP
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TVDRLRGIADSLGLEKDPAIHDNRGFGHGKLIEELWERTVGKSLSAPTFVKDFPVQTTPLTRQHRSIP
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>Rv3600c - similar to Bacillus subtilis protein YacB TB.seq 4043041:4043856 MW:29274 SEQ ID NO:276

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VLHEVRIMLDQYWPSVPHVLIEPGVRTGIPLLVDNPKEVGADRIVNCLAAYDRFRKAAIVVDFGSSICV
DVVSAKGEFLGGAIAPGVQVSSDAAAARSAALRRVELARPRSVVGKNTVECMQAGAVFGFAGLVDG
LVGRIREDVSGFSVDHDVAIVATGHTAPLLLPELHTVDHYDQHLTLQGLRLVFERNLEVQRGRLKTAR

>Rv3606c folK 7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase TB.seq 4048181:4048744 MW:20732 SEQ ID NO:277

MTRVVLSVGSNLGDRLARLRSVADGLGDALIAASPIYEADPWGGVEQGQFLNAVLIADDPTCEPREWLRRAQEFERAAGRVRGQRWGPRNLDVDLIACYQTSATEALVEVTARENHLTLPHPLAHLRAFVLIPWIAVDPTAQLTVAGCPRPVTRLLAELEPADRDSVRLFRPSFDLNSRHPVSRAPES

>Rv3607c folX may be involved in folate biosynthesis TB.seq 4048744:4049142 MW:14553

MADRIELRGLTVHGRHGVYDHERVAGQRFVIDVTVWIDLAEAANSDDLADTYDYVRLASRAAEIVAGPPRKLIETVGAEIADHVMDDQRVHAVEVAVHKPQAPIPQTFDDVAVVIRRSRRGGRGWVVPAGGAV

>Rv3608c folP dihydropteroate synthase TB.seq 4049138:4049977 MW:28812 SEQ ID NO:278

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>Rv3609c folE GTP cyclohydrolase I TB.seq 4049977:4050582 MW:22395 SEQ ID NO:279

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>Rv3610c ftsH inner membrane protein, chaperone TB.seq 4050601:4052880 MW:81987

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>Rv3671c - TB.seq 4112322:4113512 MW:40722 SEQ ID NO:280

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>Rv3682 ponA2 TB.seq 4121913:4124342 MW:84637 SEQ ID NO:281

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>Rv3721c dnaZX DNA polymerase III,[gamma] (dnaZ) and t (dnaX) TB.seq 4164995:4166728 MW:61892 SEQ ID NO:282

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>Rv3783 - TB.seq 4229255:4230094 MW:32337 SEQ ID NO:283

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FAHNIVIYFVIAIIFPKPWSWADLSFLPALALIFLNCVWVSLCFGILATRYRDIGPLLFSVVQLLFFMTPII
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ARVPYWV

>Rv3789 - TB.seq 4235371:4235733 MW:13378 SEQ ID NO:284

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>Rv3790 - TB.seq 4235776:4237158 MW:50164 SEQ ID NO:285

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>Rv3791 - TB.seq 4237162:4237923 MW:27470 SEQ ID NO:286

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>Rv3794 embA TB.seq 4243230:4246511 MW:115694 SEQ ID NO:287

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>Rv3795 embB TB.seq 4246511:4249804 MW:118023 SEQ ID NO:288

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>Rv3834c serS seryl-tRNA synthase TB.seq 4307655:4308911 MW:45293 SEQ ID NO:289
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HLELGESLGLIDMQRGAKVSGSRFYFLTGRGALLQLGLLQLALKLAVDNGFVPTIPPVLVRPEVMVGT
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>Rv3907c pcnA polynucleotide polymerase TB.seq 4391631:4393070 MW:53057 SEQ ID NO:290
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VRAAIEEMAPQLARISAERVAAELDKLLVGEDPAAGIDLMVQSGMGAVVLPEIGGMRMAIDEHHQHK
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TTELLSWWKSRGNR

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for identifying a nucleic acid or a polypeptide sequence that may be a target for a drug comprising the following steps:

(a) providing a first nucleic acid or a polypeptide sequence that is known to be a drug target;

(b) providing at least one algorithm selected from the group consisting of a "domain fusion" method, a "phylogenetic profile" method and a "physiologic linkage" method, wherein the algorithm is capable analyzing a functional relationship between nucleic acid or polypeptide sequences; and

(c) comparing the first nucleic acid or the polypeptide drug target sequence to a plurality of sequences using at least one of the algorithms as set forth in step (b) to identify a second sequence that has a functional relationship to the first sequence, thereby identifying a nucleic acid or a polypeptide sequence that may be a target for a drug .

2. A method for identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism comprising the following steps:

(a) providing a first nucleic acid or a polypeptide sequence that is known to be essential for the growth or viability of an organism;

(b) providing at least one algorithm capable analyzing a functional relationship between nucleic acid or polypeptide sequences selected from the group consisting of a "domain fusion" method, a "phylogenetic profile" method and a "physiologic linkage" method; and

(c) comparing the first nucleic acid or the polypeptide sequence to a plurality of sequences using at least one of the algorithms as set forth in step (b) to identify a second sequence that has a functional relationship to the first sequence, thereby identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism.

3. The method of claim 1 or claim 2, wherein the drug is an anti-microbial drug.

4. The method of claim 1 or claim 2, wherein the first nucleic acid or a polypeptide sequence is derived from a pathogen.

5. The method of claim 4, wherein the pathogen is a microorganism.

6. The method of claim 1 or claim 2, wherein the microorganism is *Mycobacterium tuberculosis* (MTB).

7. The method of claim 1 or claim 2, wherein the plurality of sequences used to identify a second sequence comprises a database of the gene sequences of an entire genome of an organism.

8. The method of claim 1 or claim 2, wherein the plurality of sequences used to identify a second sequence comprises a database of the gene sequences derived from a pathogen.

9. The method of claim 1 or claim 2, wherein the "phylogenetic profile" method algorithm comprises

(a) obtaining data, comprising a list of proteins from at least two genomes;

(b) comparing the list of proteins to form a protein phylogenetic profile for each protein, wherein the protein phylogenetic profile indicates the presence or absence of a protein belonging to a particular protein family in each of the at least two genomes based on homology of the proteins; and

(c) grouping the list of proteins based on similar profiles, wherein proteins with similar profiles are indicated to have a functional relationship.

10. The method of claim 9, wherein the phylogenetic profile is in the form of a vector, matrix or phylogenetic tree.

11. The method of claim 9, comprising determining the significance of homology between the proteins by computing a probability (p) value threshold.

12. The method of claim 11, wherein the probability is set with respect to the value 1/NM, based on the total number of sequence comparisons that are to be performed, wherein N is the number of proteins in the first organism's genome and M in all other genomes.

13. The method of claim 9, wherein the presence or absence is by calculating an evolutionary distance.

14. The method of claim 13, wherein the evolutionary distance is calculated by:

(a) aligning two sequences from the list of proteins;

(b) determining an evolution probability process by constructing a conditional probability matrix: p(aa→aa'), where aa and aa' are any amino acids, said conditional probability matrix being constructed by converting an amino acid substitution matrix from a log odds matrix to said conditional probability matrix;

(c) accounting for an observed alignment of the constructed conditional probability matrix by taking the product of the conditional probabilities for each aligned pair during the alignment of the two sequences, represented by $P(p)=\prod_n p(aa_n \rightarrow aa'_n)$; and

(d) determining an evolutionary distance α from powers equation $p'=p^{\alpha}(aa \rightarrow aa')$, maximizing for P.

15. The method of claim 14, wherein the conditional probability matrix is defined by a Markov process with substitution rates, over a fixed time interval.

16. The method of claim 14, where the conversion from an amino acid substitution matrix to a conditional probability matrix is represented by:

$$P_B(i \rightarrow j) = p(j)2^\wedge\frac{\text{BLOSUM62}_{ij}}{2},$$

where BLOSUM62 is an amino acid substitution matrix, and P(i→j) is the probability that amino acid *i* is replaced by amino acid *j* through point mutations according to BLOSUM62 scores.

17. The method of claim 16, where *Pj*'s are the abundances of amino acid *j* and are computed by solving a plurality of linear equations given by the normalization condition that:

$$\sum_{i} P_B(i \rightarrow j) = 1 .$$

18. The method of claim 1 or claim 2, wherein the "physiologic linkage" method algorithm identifies proteins and nucleic acids that participate in a common functional pathway.

19. The method of claim 1 or claim 2, wherein the "physiologic linkage" method algorithm comprises identifies proteins and nucleic acids that participate in the synthesis of a common structural complex.

20. The method of claim 1 or claim 2, wherein the "physiologic linkage" method algorithm comprises identifies proteins and nucleic acids that participate in a common metabolic pathway.

21. The method of claim 1 or claim 2, wherein the "domain fusion" method algorithm comprises

(a) aligning a first primary amino acid sequence of multiple distinct non-homologous polypeptides to second primary amino acid sequence of a plurality of proteins; and

(b) for any alignment found between the first primary amino acid sequences of all of such multiple distinct non-homologous polypeptides and at least one protein of the second primary amino acid sequences, outputting an indication identifying the aligned second primary amino acid sequence as an indication of a functional link between the aligned first and second polypeptide sequences.

22. The method of claim 21, wherein the aligning is performed by an algorithm selected from the group consisting of a Smith-Waterman algorithm, Needleman-Wunsch algorithm, a BLAST algorithm, a FASTA algorithm, and a PSI-BLAST algorithm.

23. The method of claim 21, wherein the multiple distinct non-homologous polypeptides are obtained by translating a nucleic acid sequence from a genome database.

24. The method of claim 21, wherein the plurality of proteins have a known function.

25. The method of claim 21, wherein at least one of the multiple distinct non-homologous polypeptides has a known function.

26. The method of claim 21, wherein at least one of the multiple distinct non-homologous polypeptides has an unknown function.

27. The method of claim 21, wherein the alignment is based on the degree of homology of the multiple distinct non-homologous polypeptides to the plurality of proteins.

28. The method of claim 21, further comprising determining the significance of the aligned and identified second primary amino acid sequence by computing a probability (p) value threshold.

29. The method of claim 28, wherein the probability threshold is set with respect to the value 1/NM, based on the total number of sequence comparisons that are to be performed, wherein N is the number of proteins in a first organism's genome and M in all other genomes.

30. The method of claim 21, further comprising filtering excessive functional links between one first primary amino acid sequence of multiple distinct non-

homologous polypeptides and an excessive number of other distinct non-homologous polypeptides for any alignment found between the first primary amino acid sequences of the distinct non-homologous polypeptides and at least one of the second primary amino acid sequences of the plurality of proteins.

31. A computer program product, stored on a computer-readable medium, for identifying a nucleic acid or a polypeptide sequence that may be a target for a drug, the computer program product comprising instructions for causing a computer system to be capable of:

(a) inputting a first nucleic acid or a polypeptide sequence that is known to be a drug target;

(b) accessing at least one algorithm capable analyzing a functional relationship between nucleic acid or polypeptide sequences selected from the group consisting of a "domain fusion" method, a "phylogenetic profile" method and a "physiologic linkage" method; and

(c) comparing the first nucleic acid or the polypeptide drug target sequence to a plurality of sequences using at least one of the algorithms set forth in step (b) to identify a second sequence that has a functional relationship to the first sequence and generating an output identifying a nucleic acid or a polypeptide sequence that may be a target for a drug .

32. A computer program product, stored on a computer-readable medium, for identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism, the computer program product comprising instructions for causing a computer system to be capable of:

(a) providing a first nucleic acid or a polypeptide sequence that is known to be essential for the growth or viability of an organism;

(b) accessing at least one algorithm capable analyzing a functional relationship between nucleic acid or polypeptide sequences selected from the group consisting of a "domain fusion" method, a "phylogenetic profile" method and a "physiologic linkage" method; and

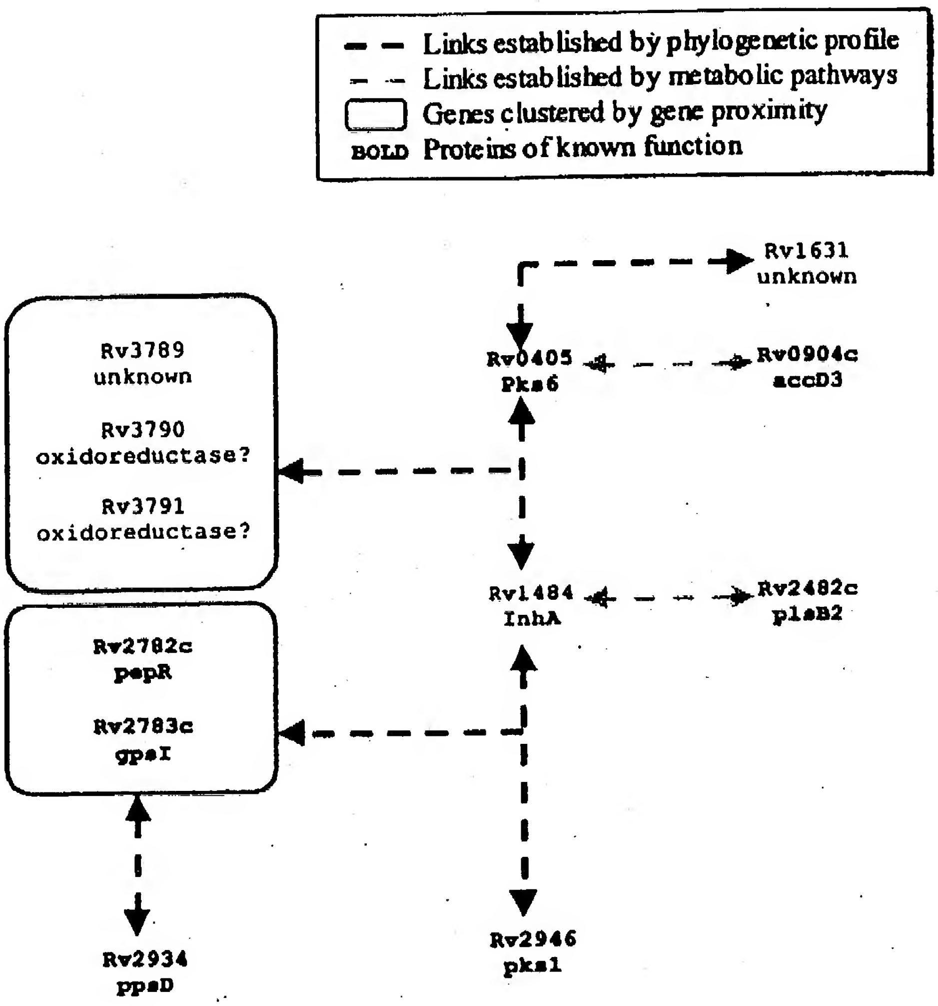
(c) comparing the first nucleic acid or the polypeptide sequence to a plurality of sequences using at least one of the algorithms set forth in step (b) to identify a second sequence that has a functional relationship to the first sequence and generating an output identifying a nucleic acid or a polypeptide sequence that may be essential for the growth or viability of an organism.

33. A computer system, comprising:

(a) a processor; and

(b) a computer program product as set forth in claim 31 or claim 32.

Figure 1


Links established by phylogenetic profile
Links established by metabolic pathways
Genes clustered by gene proximity
BOLD Proteins of known function
Rv1631
unknown
Rv3789
unknown
Rv3790
oxidoreductase?
Rv3791
oxidoreductase?
Rv0405
Pks6
Rv0904c
accD3
Rv1484
InhA
Rv2482c
plsB2
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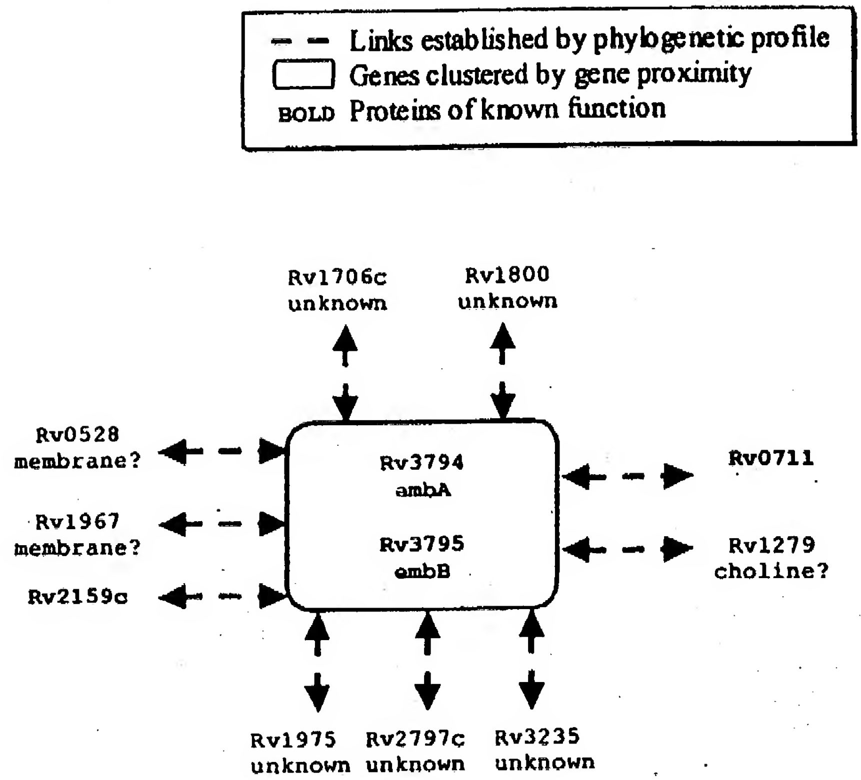
pks1

Figure 2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Genes clustered by gene proximity
BOLD Proteins of known function
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Rv1800 unknown
Rv0528 membrane?
Rv1967 membrane?
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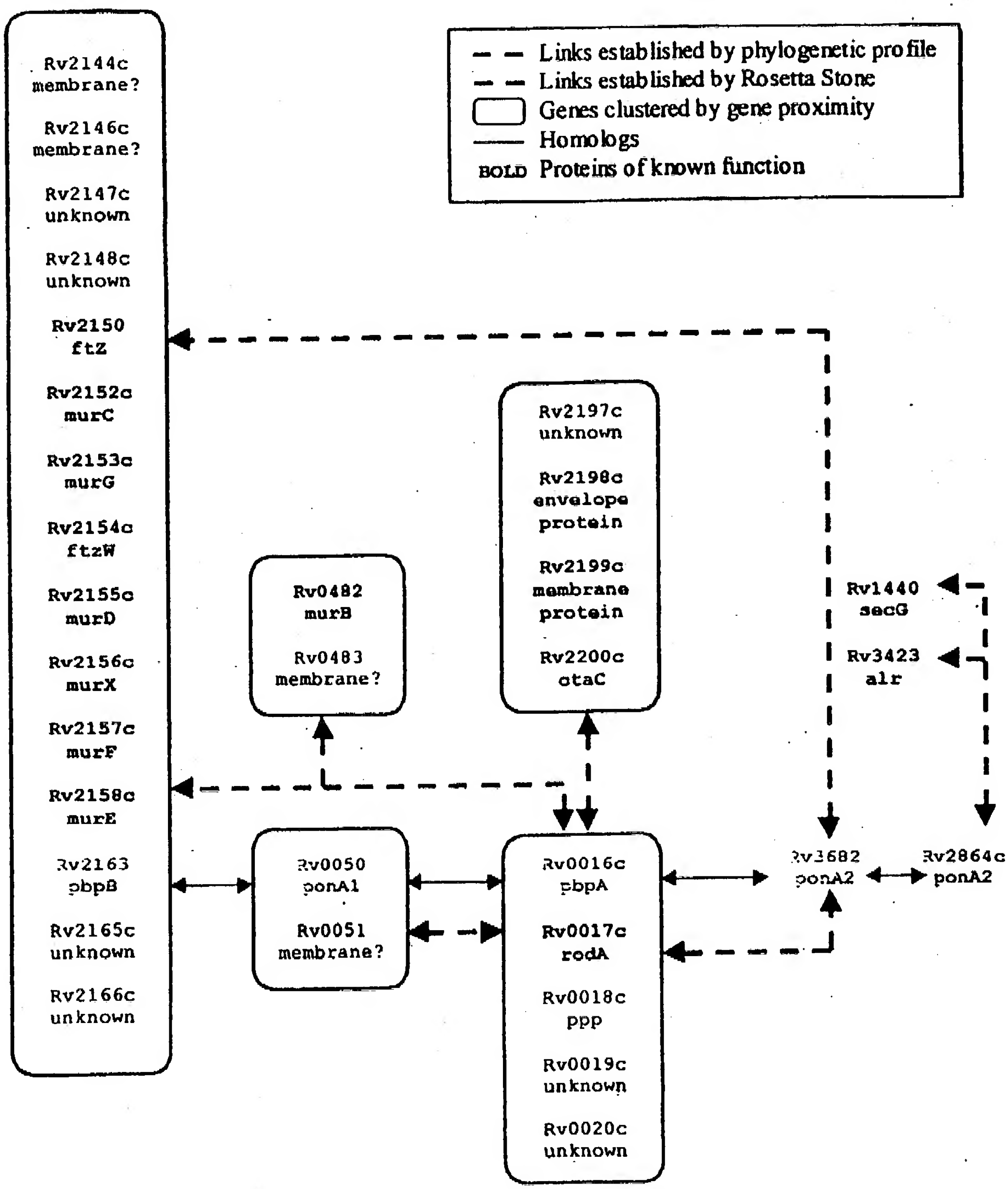
Rv0711
Rv1279 choline?
Rv1975 unknown
Rv2797c unknown
Rv3235 unknown

Figure 3


Links established by phylogenetic profile
Links established by Rosetta Stone
Genes clustered by gene proximity
Homologs
BOLD Proteins of known function
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Rv2147c unknown
Rv2148c unknown
Rv2150 ftZ
Rv2152c murC
Rv2153c murG
Rv2154c ftzW
Rv2155c murD
Rv2156c murX
Rv2157c murF
Rv2158c murE
Rv2163 pbpB
Rv2165c unknown
Rv2166c unknown
Rv2197c unknown
Rv2198c envelope protein
Rv2199c membrane protein
Rv2200c ctaC
Rv0482 murB
Rv0483 membrane?
Rv0050 ponA1
Rv0051 membrane?
Rv0016c pbpA
Rv0017c rodA
Rv0018c ppp
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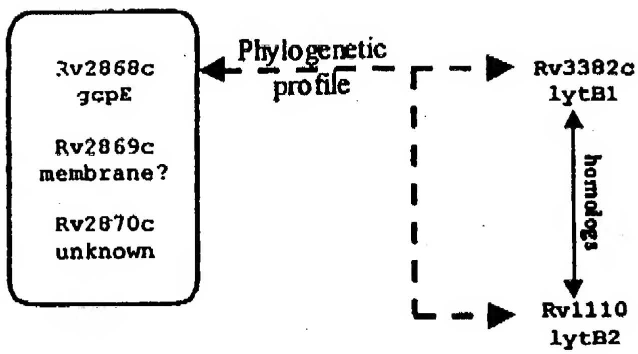
Rv0020c unknown
Rv1440 secG
Rv3423 alr
Rv3682 ponA2
Rv2864c ponA2

Figure 4


Rv2868c
gcpE
Rv2869c
membrane?
Rv2870c
unknown
Phylogenetic profile
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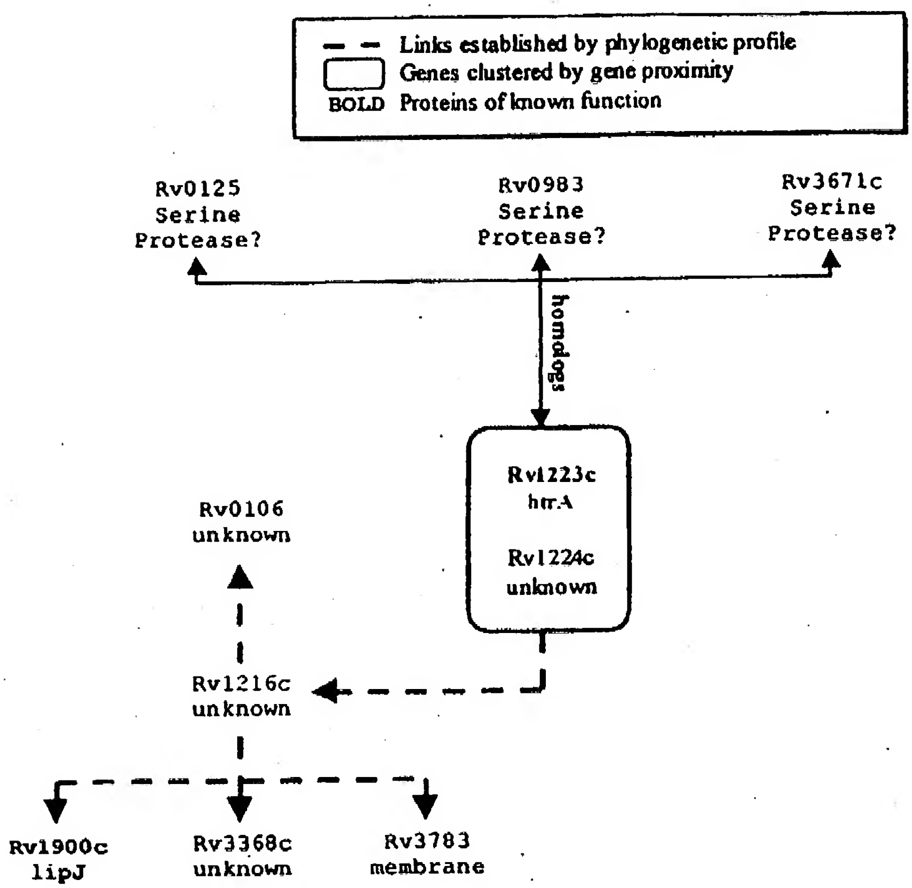
lytB1
homologs
Rv1110
lytB2

Figure 5


Links established by phylogenetic profile
Genes clustered by gene proximity
BOLD Proteins of known function
Rv0125
Serine
Protease?
Rv0983
Serine
Protease?
Rv3671c
Serine
Protease?
homologs
Rv1223c
htrA
Rv1224c
unknown
Rv0106
unknown
Rv1216c
unknown
Rv1900c
lipJ
Rv3368c
unknown
Rv3783
membrane

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/31152

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :G06F 19/00
US CL :702/19
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 702/19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN ON LINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARCOTTE et al " A COMBINED ALGORITHM FOR GENOMEWIDE PREDICTION OF PROTEIN FUNCTION" NATURE GB, 04 November 1999, vol. 402, no. 6757, pages 83-86	1-33
X	PELLEGRINI M et al. "Assigning protein functions by comparative genome analysis: protein phylogenetic profiles". April 1999, vol. 96, pages 4285-4288; the whole document	1-33
X	ENRIGHT A J et al. Protein interaction maps for complete genomes based on gene fusion elements. Nature, 04 November 1999, Vol. 402, pages 86-90, the whole document	1-33
X	TATUSOV R L et al. A Genomic perspective on protein families. Science, 24 October 1997, vol. 278, pages 631-637, the whole document.	1-33

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 FEBRUARY 2001	11 APR 2001

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	MICHAEL BORIN
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARÇOTTE E et al. Detecting protein function and protein-protein interactions from genome sequences. Science, 30 July 1999, vol. 285, pages 751-753, the whole document	1-33
A	EISEN M et al. Cluster analysis amd display of genome-wide expression patterns. Proceedings of Natl. Acad. Sci., USA, December 1998, vol. 95, pages 14683-14868.	1-33
X,P	WO 00/45322 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA), 03 August 2000, claims 1-77.	1-33